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MECHANISM OF ELECTROMAGNETIC ENERGY EFFECTS OF THE NERVOUS SYST--ETC(U)
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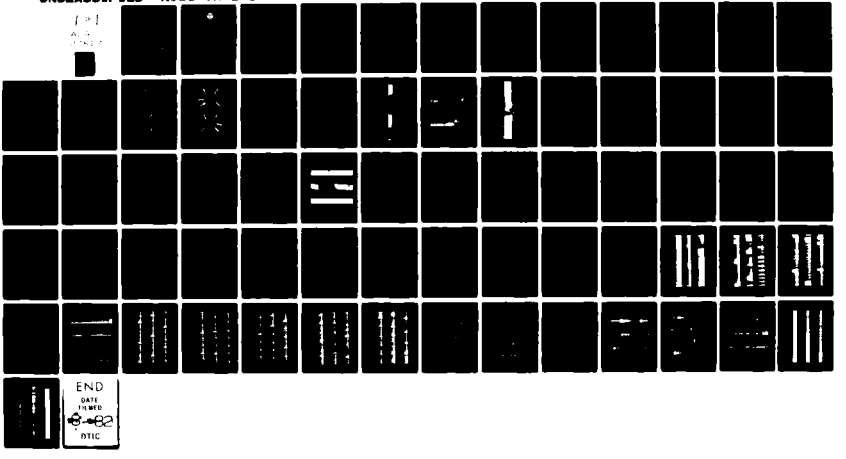

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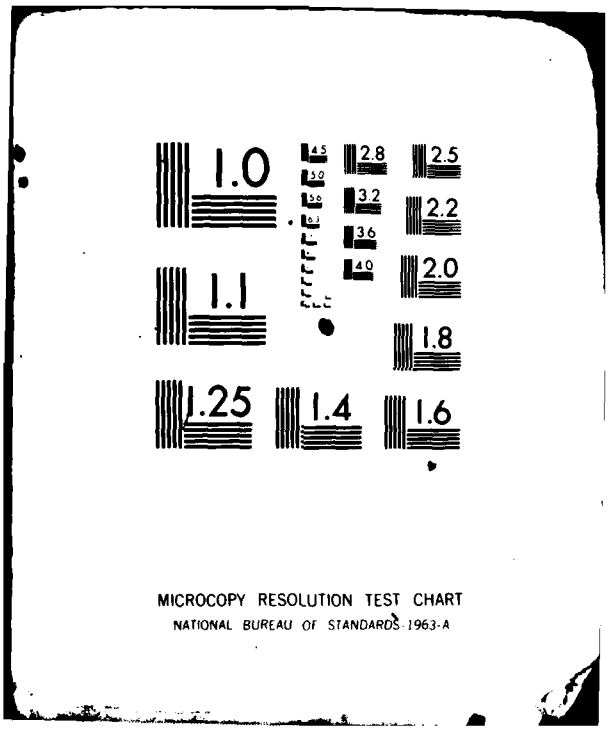
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Technical Report 698

MECHANISM OF ELECTROMAGNETIC ENERGY EFFECTS ON THE NERVOUS SYSTEM

Experimental System and Preliminary Results

N.L. Campbell
C.L. Brandt

1 July 1982

Prepared for
NOSC IR/IED Program

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ADMINISTRATIVE INFORMATION

The work reported here was performed by members of the Bioengineering Branch (NOSC Code 5123), under the direction of W. T. Rasmussen, with funding provided by the NOSC IR/IED program under program element 61152N, project ZR0000101, task area 512-ZS32. Principal investigators for this work were N.L. Campbell and C.L. Brandt; experimentation and data collection were assisted by M.C. McEuen and M.C. Teixeira-Peters (WESTEC Services, Inc).

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Effects of high-level electromagnetic (EM) energy on living nerve tissue were assessed by subjecting individual nerve cells of the marine gastropod *Aplysia californica* to microwave radiation levels of 10–300 mW/cm^2. Of the 84 cells exposed, five showed inhibitory responses and one showed excitatory response that required from 6 to 82 minutes to develop. In each case, the cell returned to normal activity within 70 minutes of removing the EM field. It appears from the data collected that interaction of EM energy with the electrical activity of nerve cells does exist, and that this interaction is not heat-related. Understanding the mechanism of this interaction will provide a valuable tool in determining the hazards of nonionizing EM energy and the necessary safety limits of exposure.

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PROBLEM

Investigate the effects of electromagnetic energy on living nerve tissue by subjecting individual nerve cells of the marine gastropod *Aplysia californica* to levels of 10–300 mW/cm^2 at 2.45-GHz microwave radiation.

RESULTS

Active nerve cells were maintained *in vitro* to observe the effects of long (up to 10 hours) periods of microwave radiation on each cell's electrical behavior. Of the 84 cells exposed, five showed inhibitory responses and one showed an excitatory response that required from 6 to 82 minutes to develop. In each case, the cell returned to normal activity within 70 minutes of removing the EM field. It appears from the data that interaction of EM energy on the electrical activity of nerve cells does exist, and that this interaction is not heat-related. Examinations of the recordings from the 15 cells used for controls indicates that most of the cells do not maintain a regular firing pattern through the experiment, which makes the identification of a response difficult. In the experiments, a response was claimed only in the most obvious cases of normal/abnormal/normal behavior coinciding approximately with the baseline/exposure/post-exposure periods. This criterion, however, assumes that the responding cell will recover in the post-exposure period, which is probably not a valid assumption, so that many responding cells may have been ignored.

RECOMMENDATIONS

A more stringent analysis method needs to be employed, either to compare control versus experimental firing patterns by means of computer statistical analysis, or to examine a less time-variable aspect of neuronal electrical activity, such as membrane ionic conductances. Microwave-induced changes in membrane ionic conductances can be directly measured by using voltage clamping (VC) techniques. Further research utilizing the experimental system described in this report should examine the possibility that effects may be more frequently observed at power densities lower than those used in these experiments.

CONTENTS

FIGURES

FIGURES (cont'd)

TABLES

SYMBOLS

Ag-AgCl	Silver-silver chloride
Ca^{++}	Calcium ion
Cl^{-}	Chloride ion
EM	Electromagnetic
E_r	Reversal potential
K^{+}	Potassium ion
KCl	Potassium chloride
Na^{+}	Sodium ion
TED	Thermoelectric device
VC	Voltage clamping
VPN	Ventral photoresponsive neuron

1. INTRODUCTION

1.1 PROBLEM

The Navy's increasing utilization of high-powered electrical equipment for command, control, and communication, as well as for surveillance and fire control, causes the exposure of military and civilian populations to rising levels of electromagnetic (EM) energy. Long-term or continuous exposure to these escalating levels of EM energy increases the potential for physiological problems. An understanding of the mechanism of the EM energy's effect on biological systems will aid in determining the hazards and safe levels of EM energy.

1.2 BACKGROUND

Microwave energy is nonionizing, and therefore is considered to be nonhazardous except at exposure levels that cause heating. Because of these obvious thermal effects, a safety limit of 10 mW/cm^2 was established to protect personnel. However, even this limit may be too high. Thermal damage is still seen to occur in organs having low blood circulation, such as the testes and the eye, following repeated exposure to less than 10 mW/cm^2 (ref 1,2). Other claimed EM effects include behavioral, neurological, hematological, and genetic changes.

Much of the research to date indicates that there are biological effects from exposure to low levels of EM energy. The Soviets fear that the major effect of EM exposure is on the nervous system (ref 3,4), and have set their safety limit to 10 $\mu W/cm^2$. With respect to neurological effects, behavioral or morphological changes have been studied. Soviet investigators claim effects on the conditioned response of various laboratory animals following exposure to less than 10 mW/cm^2 of 3-GHz or higher frequency (ref 3). Some research has detected EEG alterations following low-level EM radiation (ref 3–6). Low-level EM exposure

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1. Justesen, D.R., Diathermy versus the Microwaves and Other Radio Frequency Radiation: A Rose by Another Name is a Cabbage, Radio Science, 12(3):355, 1977.
2. Zaret, M.M., et al, Clinical Microwave Cataracts, in Biological Effects and Health Implications of Microwave Radiation, Symposium Proceedings, S.F. Cleary (ed), US Dept of Health, Education and Welfare, 1970.
3. Baranski, S., and P. Czerski, Biological Effects of Microwaves, Dowden, Hutchinson, and Ross, Inc, Stroudsburg, PA, 1976.
4. Takashima, S., B. Onaral, and H.P. Schwan, Effects of Modulated RF Energy on the EEG of Mammalian Brains. II. Appearance of Fast and Slow Waves After Chronic Irradiation, in Symposium on Electromagnetic Fields in Biological Systems, S.S. Stuchly (ed), International Microwave Power Institute, Edmonton, Alberta, 1979.
5. Servantie, B., A.M. Servantie, and J. Etienne, Synchronization of Cortical Neurons by a Pulsed Microwave Field as Evidenced by Spectral Analysis of Electrocorticograms from the White Rat, Annals of New York Acad of Sciences, 247:82, 1975.
6. Bawin, S.M., et al, Reinforcement of Transient Brain Rhythms by Amplitude-Modulated VHF Fields, in Biologic and Clinical Effects of Low Frequency Magnetic and Electrical Fields, J.G. Llaruado, et al (eds), Charles C. Thomas, Springfield, IL, 1974.

has been seen to cause cytophathology in hypothalamic and subthalamic neurons of the Chinese hamster (ref 7,8), a change in the number of dendrites in the brains of various animals (ref 3,9), and a decrease in the acetylcholinesterase activity in rats, mice, and rabbits (ref 3).

As yet, few investigators have looked at the simpler single-neuron models. Examination of the frog's sciatic nerve showed changes in conductivity rate and spike amplitude following exposure to low-power-density EM waves (ref 3). Wachtel, et al, observed an altered firing pattern of some constantly firing pacemaker cells in *Aplysia californica* on low-level exposure to 1.5- and 2.45-GHz waves (ref 10,11). Further investigation and understanding of the influence of EM radiation on nervous tissues should involve additional simple neurological models. These models allow direct measurements of individual nerve cell responses and eliminate the ambiguity of deciphering behavioral or EEG changes that involve millions of interacting cells. The marine gastropod *Aplysia* is a useful species for further examination because of (1) its well mapped ganglia (clusters of nerve cells); (2) the current knowledge regarding the function and mechanism of activity of many of its neurons; and (3) the ease of maintaining functional nerve cells *in vitro* (ref 12-15). Knowing how these cells function will be helpful in identifying the mechanism of an EM effect.

1.3 APPROACH

Single nerve cell studies are limited by the *in vitro* life span of the cells. This short life span necessitates quick experiments (as was the case in Wachtel's study) and does not allow the observation of effects manifested by long-term exposure to low-level EM energy. The goal of this study, then, was to develop an experimental system capable of

1. Sustaining the life of a nerve cell *in vitro* for up to 12 hours.
2. Monitoring the electrical activity of the cell for up to 12 hours.
3. Irradiating a ganglion with controlled levels of EM energy for long periods of time without creating artifact in the measuring electrodes.
4. Eliminating or greatly reducing the thermal effect of EM energy.
5. Collecting and analyzing the electrical activity of the neuron before, during, and after irradiation.

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7. Albert, E., and M. DeSantis, Do Microwaves Alter the Nervous System Structure? New York Acad of Sciences, 247:87, 1975.
8. Albert E., and M. DeSantis, Histological Observations on Central Nervous System, HEW (FDA) 77-8010, 11:299, 1976.
9. Frey, A.H., Behavioral Biophysics, Psych Bull, 63:322, 1965.
10. Wachtel, H., et al, Effects of Low-Intensity Microwaves on Isolated Neurons, New York Acad of Sciences, 247:46, 1975.
11. Seaman, R.L., and H. Wachtel, Slow and Rapid Responses to CW and Pulsed Microwave Radiation by Individual *Aplysia* Pacemakers, J of Microwave Power, 13(1):77, 1978.
12. Frazier, W.T., et al, Morphology and Functional Properties of Identified Neurons in the Abdominal Ganglion of *Aplysia californica,* J Neurophys, 30:1288, 1967.
13. Andresen, M.D., and A.M. Brown, Photoresponses of a Sensitive Extraretinal Photoreceptor in *Aplysia,* J of Physiol (London), 287:267, 1979.
14. Brown, A.M., and H.M. Brown, Light Response of a Giant *Aplysia* Neuron, J Gen Physiol, 62:239, 1973.
15. Kandel, E.R., Cellular Basis of Behavior, W.H. Freeman and Co, San Francisco, CA, 1976.

2. EXPERIMENTAL SYSTEM

A major portion of the effort expended during the first year of this study was the development of an experimental system (equipment and procedures) to meet the study goal.

2.1 EQUIPMENT

NEUROELECTRICAL RECORDING EQUIPMENT

Two W-P Instruments, Inc, Model M-707 Micro-probe DC Amplifiers were acquired to monitor the intracellular electrical activity of two cells at one time. In addition to this monitoring capability, these high-impedance amplifiers had a unique, built-in Wheatstone bridge circuit, which allowed the passage of current through the same intracellular electrode that was used to monitor electrical activity. Therefore, only one electrode needed to be implanted in the cell. Current was delivered to the bridge circuit of the amplifier from a W-P Instruments, Inc, Model 1880 Dual Stimulus Isolator. The stimulus isolator was driven by a W-P Instruments, Inc, Model 302-T Dual-Channel Stimulator. The current-passing capability was used to pulse the cell, as will be described below. The output of the amplifiers was displayed on a dual-channel oscilloscope and on a four-channel chart recorder. The preamplifiers of this recorder were modified to accept the single-ended grounded output of the W-P amplifier.

The intracellular microelectrodes used in this study were glass capillary tubes pulled to a fine point (0.5 μm diameter) on a David Kopf Model 700C Vertical Pipette Puller. After pulling, the tip was bent, with the heat from the pulling coil being used to soften the glass. The tip must be bent to 90 degrees and the bent portion cannot exceed the range of 3.4 to 3.8 mm, so that it will fit within the stripline when the cover is in place. While these size restrictions were severe, microelectrodes of this type were fabricated successfully on a routine basis. After pulling and bending, the pipettes were placed on a plastic holder and immersed, tip end down, in filter-sterilized 3M KCl, where they filled overnight by capillary action.

Suitability of the microelectrodes for use in an experiment was determined by measuring the electrical resistance of the electrode when mounted in series with two Ag-AgCl electrodes, a KCl salt bridge, and a digital voltmeter. A resistance in the range of 4 to 7 MΩ indicated a tip diameter in the range of 0.5 μm, a size which penetrates the neurons easily with a minimum of trauma to the cells.

These microelectrodes were interfaced to the amplifier probe by a W-P Instruments, Inc, microelectrode holder. The acquired holders were modified to provide a more rigid mounting for the microelectrodes in the horizontal plane. The modification consisted of a plastic rod drilled to accept the shank of the microelectrode. Each end of the rod was threaded. One end threaded into the W-P electrode holder and, when tightened, sealed a rubber O-ring around the pipette. The other threaded end carried a threaded cap containing another O-ring. When this cap was tightened, the second O-ring was compressed around the electrode shank. Supporting the electrode shank at two points held it firmly in the horizontal position, and prevented its rotation out of the horizontal when the tip was advanced into a cell. This rigid mounting reduced the chance of microelectrode tip breakage. The electrode was inserted into the holder until contact was made with the Ag-AgCl pellet in the back of the holder. The cavity within the holder was then filled with 3M KCl to act as a KCl salt bridge.

An Ag-AgCl reference electrode was connected via a KCl salt bridge to the seawater surrounding the ganglion, and was used by both amplifiers as a ground. The microelectrodes were positioned over and gently lowered into a cell by Brinkman Model CP-V Micromanipulators which were modified to include a planetary reduction drive on the vertical axis. An AO Swing Arm Stereo Microscope was used to view the cells for proper microelectrode positioning. The vertical post on the swing arm stand was replaced with a longer one, and a new method of mounting the microscope body was devised which allowed the microscope to be swung over the irradiation fixture, so that the cells could be viewed from the top at about a 45-degree angle. A microscope lamp was modified to illuminate the surface of the cells and was equipped with a removable polaroid filter and a removable red filter.

MICROWAVE IRRADIATION EQUIPMENT

A Hewlett-Packard Model 616A uhf Signal Generator served as the microwave source. This generator output a maximum of 1 mW over the frequency range of 1.8 to 4.0 GHz, and had pulse modulation and amplitude modulation capabilities. The signal from the generator was amplified by a Hewlett-Packard Model 489A Amplifier, which was designed for the range 1 to 2 GHz. Since this study examined the effects of 2.45 GHz, the amplifier did not operate to its full capacity of a 30-dB gain. The maximum output power observed for 2.45 GHz was 400-600 mW with the use of a directional coupler, and 1000 mW without. The amplifier's output power was monitored with an H-P Model 478A Thermistor Mount and read on an H-P Model 431B Power Meter. A directional coupler diverted 1% of the forward energy to the thermistor mount.

The output of the amplifier was fed into a stripline specially designed by A.R. Hislop (NOSC, Code 9262) which can accommodate the frequency range of 2-12 GHz. A major constraint in the design of the stripline was that, as the frequency to be used was increased, the cross-sectional area of the stripline had to be decreased. Thus the highest frequency that could be studied on this project was determined by the minimum cross-sectional area presented by the ganglion, the seawater, and the microelectrodes penetrating the neurons. Another constraint on the design of the stripline was that the ventral surface of the ganglion had to be normal to the E-field to minimize absorption of energy by material between the cell and the center conductor. Figure 1 is a sketch of the irradiation fixture as seen in a cross-section view through the center of the ganglion chamber. This sketch reveals some of the unique features of the experimental system: (1) the major portion of the stripline was removable, which allowed complete freedom in viewing the ganglion, selecting the neurons to be impaled, and placing the electrodes; (2) the microelectrodes, instead of being used straight, were bent 90 degrees at the tip, which allowed the ganglion surface to be placed normal to the E-field with the electrodes' shanks penetrating the side of the stripline; (3) the ganglion chamber was very shallow, but just deep enough to accommodate the ganglion and the Sylgard base to which it was pinned, which minimized the amount of seawater that was penetrated by the microwaves before reaching the neuronal tissue; (4) the ganglion was constantly suffused with fresh, cooled seawater; and (5) the entire fixture was maintained at a constant temperature by direct mounting on a large thermoelectric device. The top and three sides of the removable stripline cover were machined from a single piece of aluminum bar stock. The inner channel was square in cross section and 14 mm on a side, with the center conductor 7 mm from the base plate. Coaxial connectors attached to each end of the center conductor. Flanges on each side of the stripline contained holes which lined up with guide pins on the base plate. Other holes

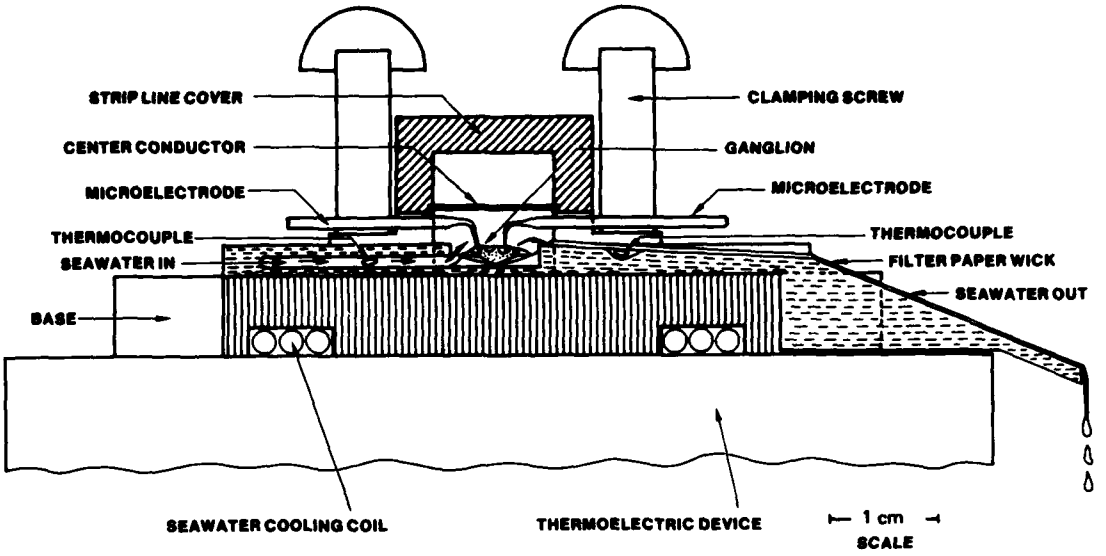

Figure 1. Cross-section diagram of irradiation fixture.

in the flanges provided ports for screws which fastened the cover to the base plate. The two sides of the cover contained notches which straddled the ganglion chamber and provided access for the electrode shanks. Three turning screws were located between the ganglion chamber and the terminal load. The screws were used to adjust reflected power to zero. The dimensions of this stripline were such that

$$P_d = \frac{P_o\,(mW)}{3.25\,(cm^2)}$$

where P_d = power density

P_o = power output from amplifier.

GANGLION MAINTENANCE EQUIPMENT

The ganglion was pinned in a chamber machined from a piece of plexiglass. Figure 2 shows a top and side view of the chamber in its present configuration (the chamber underwent a number of modifications during the course of this study). The central well had a small amount of Dow-Corning Sylgard on its floor. This material will accept and hold

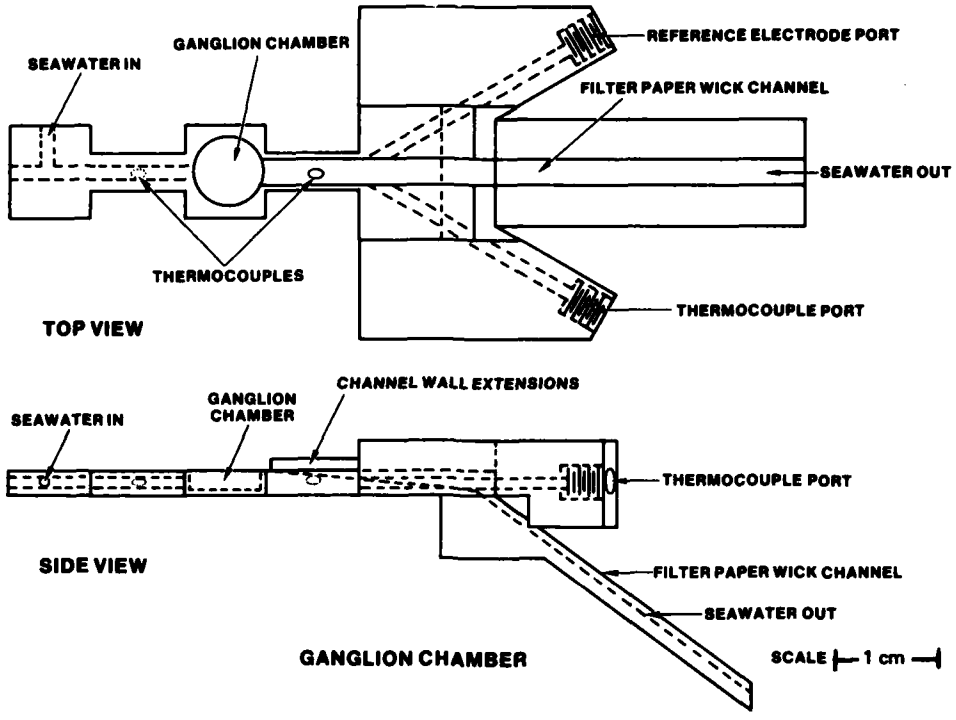

Figure 2. Ganglion chamber and flow system.

the small cactus spines used to pin the ganglion firmly into the chamber. A hole was drilled into the left side of the chamber as an inlet for the cooled seawater. The ganglion chamber was fastened to the base plate of the irradiation fixture with epoxy adhesive.

The *in vitro* life span of the neurons is increased if they are kept at a cool temperature, around 16°C. This temperature was maintained by mounting the ganglion chamber on the base plate of the stripline, and by mounting the base plate directly on a thermoelectric device (Cambion TED, Model 806-1002). A continuous flow of tapwater through the TED acted as a heat sink. A constant temperature was maintained by a Cambion Model 809-3030-01 Bipolar Temperature Controller which used a thermistor mounted in the base plate to monitor its temperature.

Many of the ganglia studied to date were bathed in the same 0.2-0.4 ml of seawater throughout the entire experiment (which sometimes lasted up to 10 hours). It was feared that a buildup of waste products from the metabolizing neurons would create undesirable effects on the behavior of the cells or on their *in vitro* life span. Many of the cells that were examined for long periods did show irregular behavior after about 4 hours of study. A peristaltic pump was incorporated into the system to increase the longevity of a healthy cell by constantly exchanging its surrounding fluid with fresh, cooled seawater, thereby eliminating waste buildup. However, the release and uptake of fluid in the ganglion chamber was inconsistent and often either overflowed, which caused a shorting of the electrical recording setup, or completely drained, which caused irregular activity in the cell being studied. This problem was overcome by containing the seawater in a completely closed

system (except at the ganglion chamber) with all air bubbles removed, and requiring only an occasional addition of a few drops of seawater to replenish the loss that resulted from evaporation over the ganglion chamber. The fluid in the chamber was then seen to pulse up and down with the action of the pump, and this caused pulsating deflections in the electrical recording. The problem was eliminated by insertion of several small reservoirs between the pump and the chamber. The reservoirs were meant to absorb and eliminate the pulse shock resulting from the pump, but the pulsations on the recordings were never completely eliminated. The major problem with the use of the pump was electrical noise on the recordings traceable to the process of constricting the tubing containing the seawater. This noise could not be eliminated, and the peristaltic pump was dropped as a means of providing seawater flow over the ganglion.

A gravity flow method of moving water over the ganglion was then developed and used. Approximately 2 liters of fresh seawater was maintained in a constant-pressure reservoir situated at a higher level than the chamber. Seawater from this reservoir then flowed into the chamber by gravitational force, the flow rate varying between 0 and 8 ml per minute (as controlled by a valve). Before flowing into the chamber, the seawater was cooled by passing it through a coil of plastic tubing lying in a raceway between the cool surface of the TED and the bottom plate of the irradiation fixture. By the time the seawater reached the ganglion, it had been cooled to the same temperature as the TED plate. A plastic channel lined with absorbent filter paper attached to the top right edge of the ganglion chamber drew the seawater out of the chamber and maintained a constant height of seawater over the ganglion. The seawater passed down this channel and was discarded.

Not only did the TED and suffusion system maintain the cells in a cool, healthy condition, but they also served to minimize any temperature increase in the cells during EM exposure. Thermocouples were inserted in the inlet channel and outlet channel of the ganglion chamber to monitor the temperature of the seawater as it entered and left the chamber. The temperature difference between these two thermocouples was measured by a portable analog microprobe thermometer (Bailey Instruments Model BAT-4), and was output on the chart recorder.

2.2 PROCEDURE

The living nerve cells whose electrical activities were monitored by this system are located in the abdominal ganglion of the marine mollusc, *Aplysia californica*. Figure 3 shows the intact and the dissected animal, as well as the location of the abdominal ganglion. A map of identified neurons in this ganglion is shown in figure 4. The animal was sacrificed and the ganglion removed and pinned to the floor of the ganglion chamber with cactus needles. When necessary, excess connective tissue was removed from the top surface of the ganglion to allow easier viewing and penetration into a cell. The removal was accomplished by using fine-tipped forceps to grip the tissue over the cells, and by using a piece of razor blade (held by a hemostat) to carefully slice the connective tissue away. Seawater was then suffused over the ganglion, usually at a rate of 5 ml per minute.

Microelectrodes were selected, inserted into electrode holders, and positioned in the seawater over the cells. The amplifier bridge was then balanced to deliver the predetermined current necessary for pulsing (see below). The seawater flow was stopped, and the level was lowered to allow accurate positioning of each electrode over the center portion of a cell body. Once the tip was positioned just over, or just touching, the cell membrane, visual observation through the microscope was no longer needed, and the suffusion of seawater

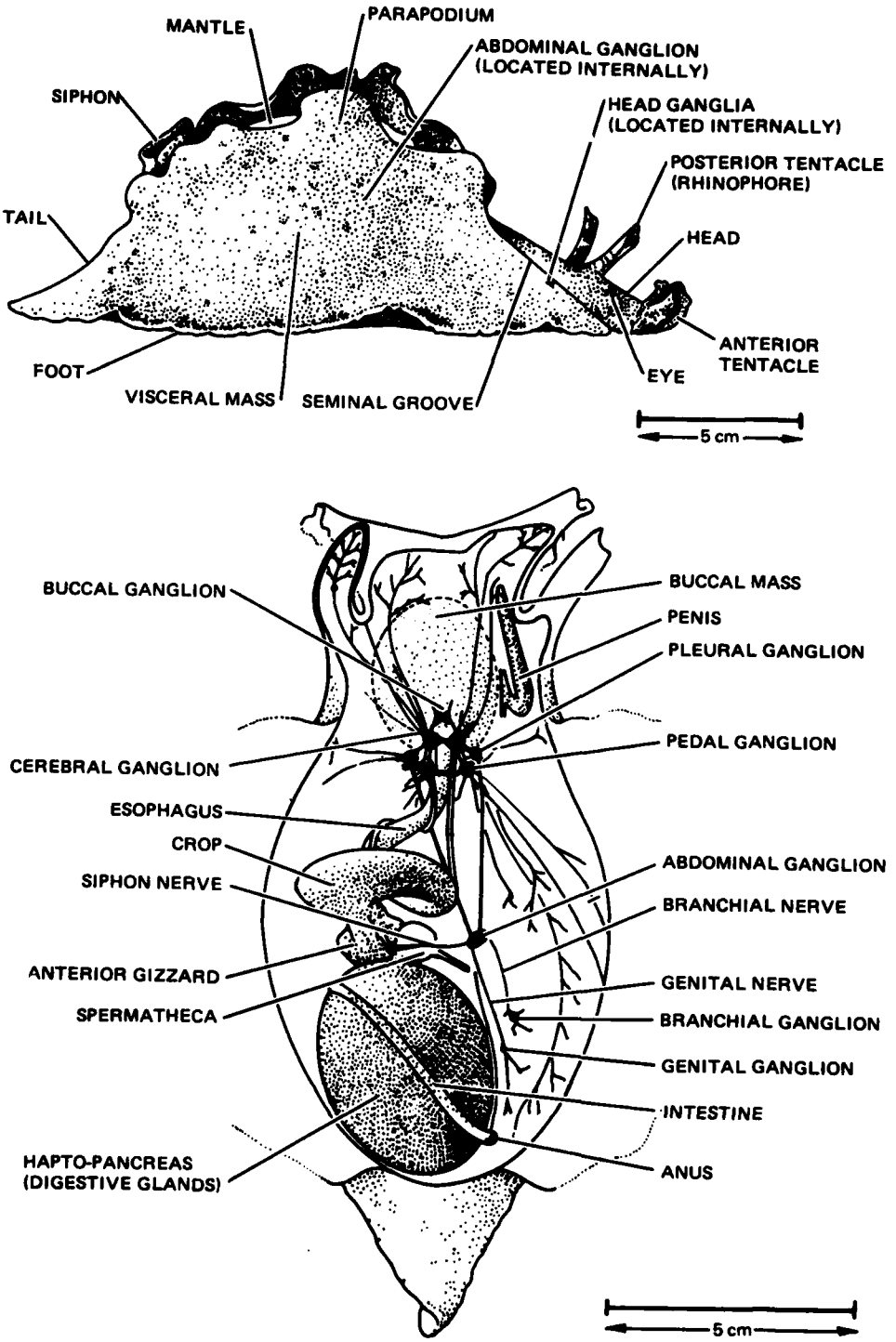

Figure 3. Side view of intact *Aplysia californica* (top) and internal organs and nervous system (bottom).

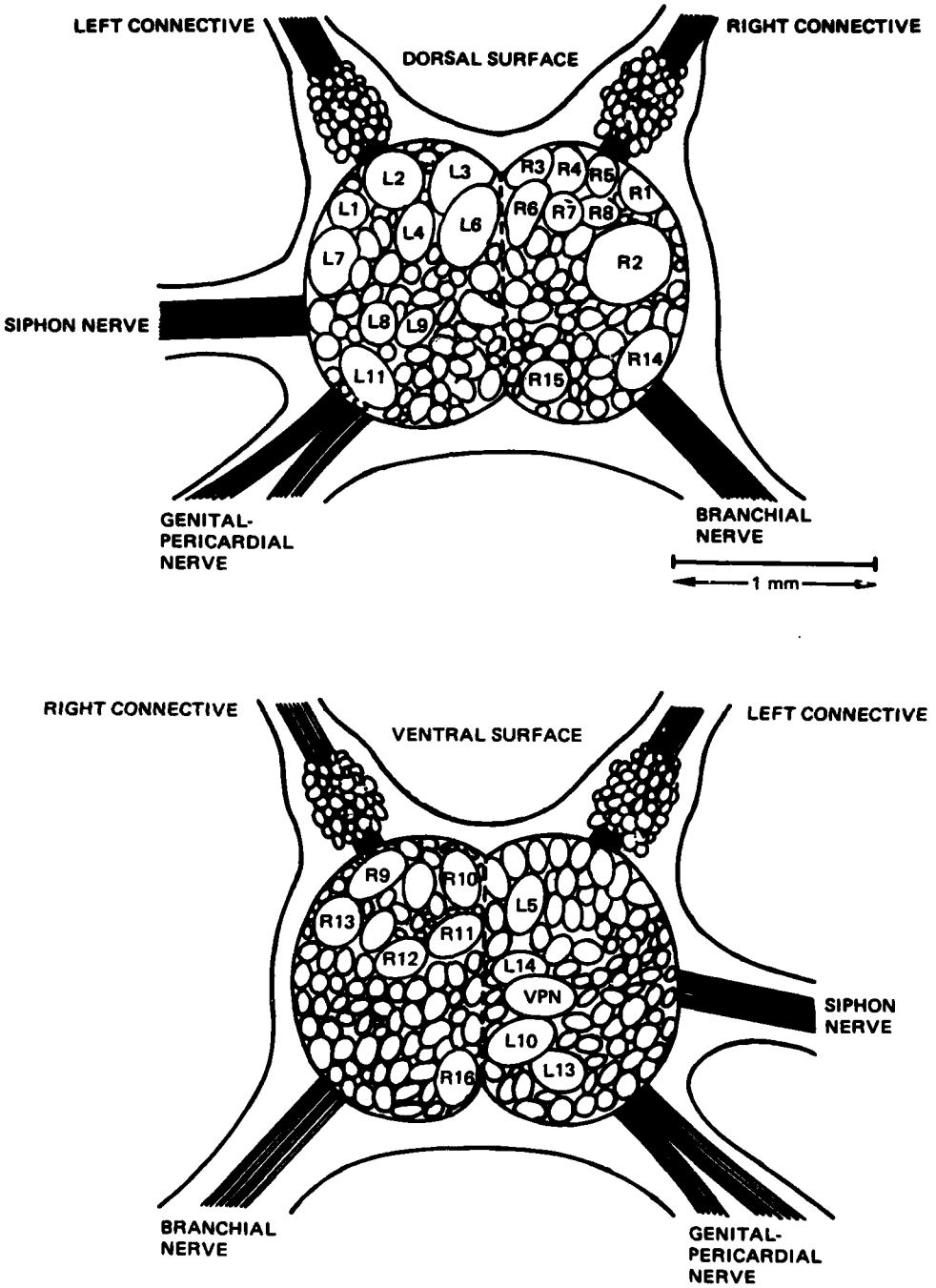

Figure 4. Map of identified neurons in the abdominal ganglion of *Aplysia californica.*

was re-established. The electrode was carefully lowered into the cell by means of the micromanipulator. Gentle tapping on the micromanipulator was usually required to push the tip of the electrode through the connective tissue and cell membrane. Successful impalement of a cell was verified by spiking activity, as seen on the oscilloscope. To seal the membrane around the penetrating electrode, and to help ease the trauma of insertion, 1-second pulses of 10-nA current were injected into the cell every 2 seconds. Pulsing was continued for approximately 30 minutes. During this time, the stripline cover was carefully placed over the ganglion and microelectrodes and bolted against the aluminum base plate.

After the pulsing period, electrophysiological data were collected for a period of approximately 1 hour. The data collected during this baseline period represented the cell's normal electrical activity. The EM energy was then turned on: 2.45 GHz, continuous wave. Initially, the output power from the amplifier was limited to about 500 mW (this varied daily). Currently, 1 W of power can be output. Although 1-W output power corresponds to more than 300 mW/cm^2, a level which should cause heating, no heating of the cells occurred in this temperature-controlled system, as observed by the behavior of the cells; ie, their pattern of electrical activity did not change when the EM field was turned on. Several experiments were performed to observe the neural response to an increase in temperature of 1°C. The cells responded immediately, usually by increasing the frequency of their electrical activity. Since the temperature control capabilities of this system seemed to counteract the heating potential of the EM energy, and since it was initially believed that a higher power density of energy should increase the frequency and intensity of any response, it was decided to use high-power-density (up to 300-mW/cm^2) exposures in these experiments. (However, as will be discussed below, the results of this study indicate that this initial assumption of high power density yielding a higher frequency of response may be incorrect.)

In the experiments to date, the EM exposure period lasted from 5 minutes to 335 minutes. If a dramatic effect occurred during exposure (such as the complete cessation of electrical activity), the field was removed. Following the exposure period, the cell's electrical activity was monitored for an additional hour during a post-irradiation period.

3. RESULTS

Results will be discussed for cells not exposed to EM energy, for cells exposed to EM energy, and for control experiments examining potential equipment-caused artifacts. Some cells could be identified by name, depending on their location in the ganglion and their electrical behavior (ref 12-15). These names are indicated in the following text and tables. Other cells could not be identified and are represented by "X". Each cell's electrical behavior is also given as one of the following:

1. Beating – repeated spikes at a consistent frequency.
2. Bursting – bursts of spikes followed by periods of silence, at a regular frequency.
3. Silent – little or no spiking.

3.1 CELLS NOT EXPOSED TO EM ENERGY

Forty-two of the cells examined were not exposed to EM energy. Data collection from these cells is tabulated in appendix A. These cells included 12 VPNs, six L10s, four R15s, one L5, and 19 unknowns, and were typed as 30 beating, nine bursting, and three silent. Many of the cells were not exposed because of problems that occurred during the experiment. One problem encountered early was excessive noise in the electrical recording. This noise was later attributed to an electrical connection (via saltwater leaking out of the chamber) between the electrode and the large aluminum base. Many of the recordings taken between 14 April 1980 and 12 May 1980 suffered from this source of noise. The noise was eliminated by fabricating a completely sealed ganglion chamber, thereby preventing leakage of the saltwater. Another noise problem arose with the use of the peristaltic pump, which affected some of the recordings between 24 August 1980 and 14 January 1981.

Some cells not exposed to EM energy were damaged by the initial insertion of the pipette. These cells never developed healthy electrical activity. The recording from one such cell studied 25 June 1980 is shown in fig 5. Despite repeated pulsing periods, this cell never developed a normal spike with an amplitude of about 70 mV. In many experiments, the pipette came out of the cell, either by accidental movement of the experimental apparatus, or by expulsion by the cell itself. In most of these cases, the pipette was reinserted by the experimenter within a few minutes of its expulsion, and with no trauma to the cell (as indicated by the cell's electrical behavior). However, in a few cases, the cell became damaged either during the expulsion or by the reinsertion of the pipette. Figure 6 shows the recording from a cell studied 4 June 1980. Accidental movement of the pipette at point A in the figure caused the pipette to come out of the cell. Following reinsertion of the pipette at point B, the cell was seen to be damaged. Expulsion of a pipette is shown in fig 7 in a cell studied 9 July 1980. At point A in this figure the pipette was coming out of the cell, and was completely expelled by point B.

One cell (examined on 27 June 1980) not exposed to EM energy was exposed to tubocurarine (curare), a synaptic inhibitor. Curare was used to isolate the cell being studied by minimizing the communication from other cells, thus simplifying the model. As can be seen in fig 8, the curare (added at point A) caused an initial short period of inhibition followed by rapid firing. Apparently, the curare eliminated the synaptic communication of one or more inhibiting neurons. Addition of curare, however, does not result in complete synaptic inhibition, and therefore the goal of isolating one cell could not be achieved by the use of curare alone.

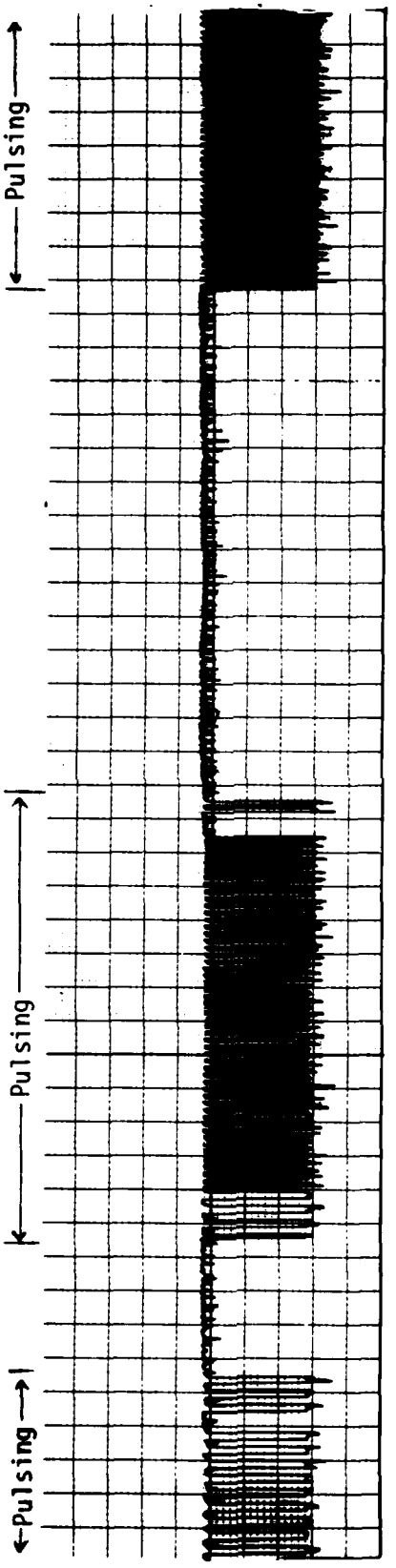

Figure 5. Damaged cell.

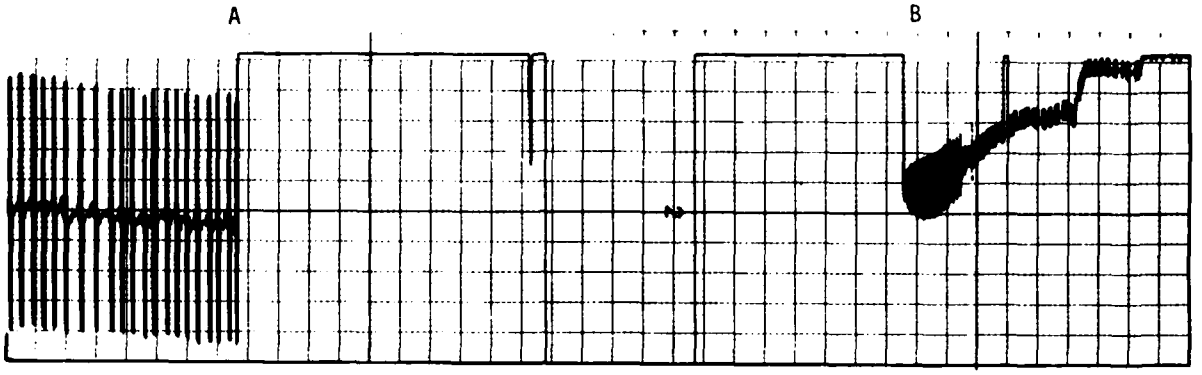

Figure 6. Accidental movement of a pipette while in a cell.

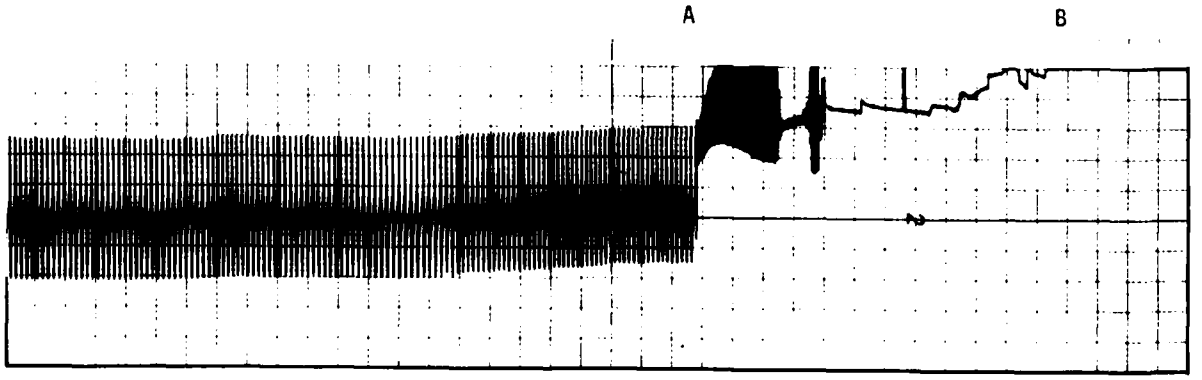

Figure 7. Expulsion of pipette by cell.

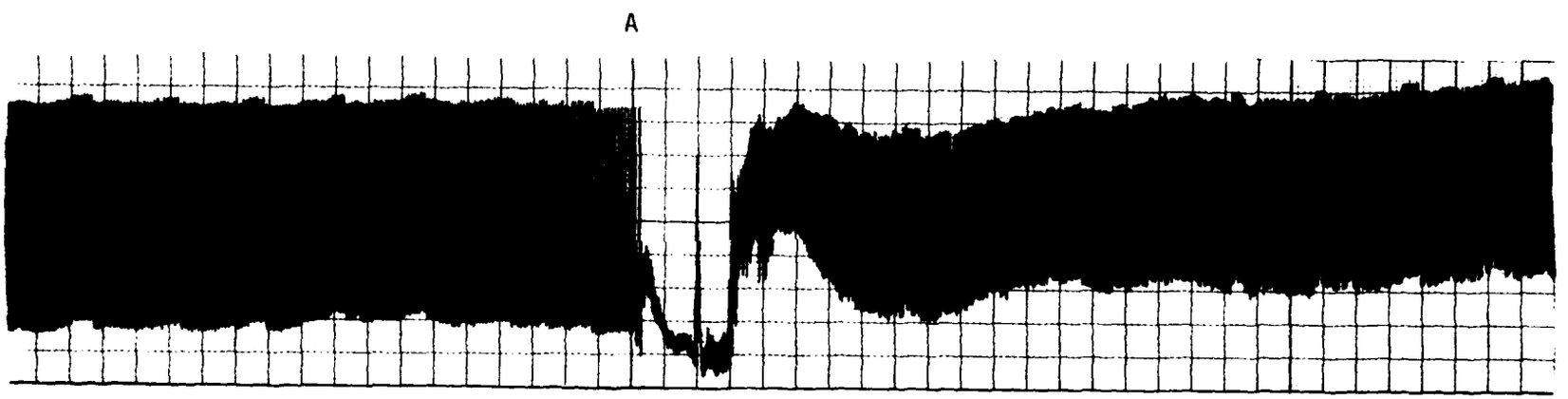

Figure 8. Effects of curare on cell studied 27 June 1980.

Fifteen cells were studied as controls to see how a cell's activity changed over long periods of time. These cells are so indicated in appendix A in the Comments column. This group of control cells contained six VPNs, four L10s, one L5, and four unknowns, which were typed as 13 beating, one bursting, and one silent. Seven control cells were pulsed for an average of 27 minutes. Baseline data collection for all the control cells averaged 269 minutes. Four of these cells [6 August 1980 (L10), 9 February 1981 (VNP and L10), and 7 April 1981 (X)] maintained a constant behavior throughout the experiment (some required an initial adjustment period). However, the majority of cells altered their behavior at some point during the experiment. Five cells [11 June 1980, 19 June 1980, 6 August 1980 (VPN), 18 August 1980, and 17 March 1981 (VPN)] showed increased irregularity over time. One cell [23 March 1981 (L5)] became more regular over time. Two cells [2 December 1980, 17 March 1981 (L10)] gradually altered their regular beating frequency. Two cells [26 June 1980, 7 April 1981 (L10)] developed a beating pattern after a long period of silence. And one cell [23 March 1981 (L10)] altered its behavior many times throughout the experiment.

3.2 CELLS EXPOSED TO EM ENERGY

Appendix B lists the data collected on 84 cells exposed to EM radiation. These cells included

29 VPNs	2 R11s	1 R16
9 L10s	2 L11s	1 L6
5 R15s	1 R2	1 L7
2 L5s	1 R14	30 Unknown (X)

and were typed as 70 beating, 10 bursting, and four silent. Forty exposed cells were pulsed for an average of 31 minutes. All of the exposed cells were examined on the average for 98 minutes during baseline, 103 minutes during exposure, 81 minutes post exposure, and 297 minutes total.

The behavior of the exposed cells was qualitatively analyzed from the chart recordings to classify the cells as

N – no response.
R – response.
P – possible response.
U – unable to decide.

Each cell's classification is shown in appendix B. To classify a cell as responding to the EM energy, development of irregular activity during exposure and recovery of normal activity post exposure were used as the criteria for labeling a change in neuronal activity as due to the radiation. Cells developing irregular activity during exposure, but not recovering following exposure, were classified as possibly showing a response, since so many of the control cells exhibited irregular behavior at some time during the experiment. Table 1 shows the breakdown of these classifications.

Classification	Cell	Type	Average Duration of Pulsing, min	Average Duration of Baseline, min	Average Exposure, mW/cm^2	Average Duration of Exposure, min	Average Duration of Post-Exposure, min	Average Total Time, min
N	10 VPN	18 beating	29	113	198	107	84	325
	2 R15	4 bursting	(n=17)	(n=23)		(n=23)	(n=23)	(n=23)
	2 L10	1 silent						
	9 X							
	23							
R	2 VPN	5 beating	23	64	120	83	136	295
	1 R2	1 silent	(n=2)	(n=6)		(n=6)	(n=6)	(n=6)
	3 X							
	6							
P	9 VPN	21 beating	35	106	159	125	88	335
	6 L10	2 bursting	(n=12)	(n=25)		(n=25)	(n=25)	(n=25)
	2 L5	2 silent						
	2 R11							
	1 R14							
	1 R16							
	1 L11							
	3 X							
	25							
U	8 VPN	24 beating	31	86	149	87	63	245
	3 R15	5 bursting	(n=9)	(n=30)		(n=30)	(n=30)	(n=30)
	1 L10	1 silent						
	1 L6							
	1 L11							
	1 L7							
	15 X							
	30							

Table 1. Classifications of cells exposed to EM energy.

NO RESPONSE

Twenty-three cells showed no observable response to the EM exposure. The electrical behavior of these cells before, during, and after radiation did not change. A few of these cells classified as "N" developed problems during the experiment (see Comments in appendix B) but the problems were determined to be minor and to have no influence on the cell's activity.

RESPONSE

Six cells appeared to respond to the microwave energy; data strips for these cells are presented in appendix C. In five cells the response was inhibitory, and in one cell the response was excitatory.

Each of these responses is shown as follows:

Cell	Date Studied	Response	Figures
VPN	18 June 1980	Inhibition	C-1–C-3
X	7 May 1980	Inhibition	C-4–C-5
X	17 July 1980	Inhibition	C-6–C-10
R2	23 September 1980	Excitation	C-11–C-13
VPN	27 January 1981	Inhibition	C-14–C-16
X	29 January 1981	Inhibition	C-17–C-18.

The numbers written above each strip in the figures represent the time, in minutes, from the initiation of the experiment.

Figures C-1 through C-3 represent several hours of recorded electrical activity of a VPN studied on 18 June 1980. Baseline data were collected for 70 minutes and showed a very regular beating pattern with occasional periods of excitation (normal for the VPN), as seen at points A and B of fig C-1c. A 5-second stimulus of visible light, known to hyperpolarize the VPN (13,14), was introduced periodically throughout this experiment. At 65 minutes after initiation of the recording (see fig C-1c), light was shown and the cell responded by hyperpolarizing, which identified it as a VPN. No other inhibitions were observed in the 70 minutes of baseline data. Seventy minutes into the experiment, the EM energy was turned on to a power density of 188 mW/cm^2. As can be seen in fig C-2a, the electrical activity of the cell initially maintained its baseline behavior. At 82 minutes, the cell underwent its first unprovoked (ie, no light stimulus) inhibition for a period of about 30 seconds. These inhibitory periods progressively became more frequent, as can be seen in fig C-2b and C-2c. The field was turned off at 160 minutes. The inhibitory periods became pronounced immediately after cessation of the radiation and completely disappeared by 230 minutes (see fig C-3). The cell did show an unexplained increase in the number of excitatory periods compared to its baseline behavior.

Figures C-4 and C-5 show the response of a cell studied 7 May 1980. This cell was exposed twice to 2.45-GHz energy at a power density of 126 mW/cm^2. The first exposure was turned on at point A, fig C-4b, and off at point B, fig C-4c. Within 6 minutes of initiation of the first exposure, the cell hyperpolarized and remained hyperpolarized until 1.5 minutes

after the field was turned off (fig C-4). The cell was allowed to recover for 32 minutes, then was exposed again to 126 mW/cm^2 (point A, fig C-5b). Within 12 minutes, the cell hyperpolarized and remained hyperpolarized for 2 minutes after the field was removed. The cell was monitored for another 80 minutes after cessation of the second exposure, and showed no further abnormalities in its beating pattern.

On 17 July 1980, another neuron showing inhibitory effects of EM exposure was observed. Figures C-6 through C-10 represent the electrical activity of this cell during the 8 hours and 20 minutes the cell was studied. This cell's behavior was strongly influenced by other cells, as can be seen by the repetitive synaptic signals in fig C-6. Point A on strip b of this figure shows a strong inhibitory synaptic input followed immediately by a strong excitatory synaptic input. Point B shows many synaptic inputs, both excitatory and inhibitory, which maintained the cell's potential below threshold (ie, prevented firing) for about 30 seconds. These communications to the cell showed a periodicity which changed slightly throughout the 8 hours and 20 minutes of study. Irradiation with 111 mW/cm^2 was started at 72 minutes and turned off at 142 minutes (point A, fig C-7c). Thirty minutes into the exposure period, the cell began to show increasing duration and frequency of inhibitory periods. This marked inhibition ceased within 30 minutes of turning the radiation off, and the cell resumed its normal baseline activity (fig C-8).

To verify that the response to the EM energy was not a thermal effect, the cell's response to changing temperatures was studied, and is shown in fig C-9. Between points A and B in fig C-9a, the temperature of the cell was increased from 16°C to 17°C by raising the temperature of the cooling plate. The immediate response of the cell to this increase in temperature was an increase in firing frequency during the beating periods (fig C-9a and C-9b). No increase in frequency or duration in inhibitory periods was seen in the cell's response to temperature increase. The cell's sensitivity to changes in temperature was further studied by decreasing the temperature from 17°C to 15°C between points C and D on fig C-9c. The cell's immediate response was to decrease its firing frequency during beating periods.

This same cell was then exposed to a second period of radiation by using a lower power density of 31 mW/cm^2. Figure C-10 shows the cell's response. Radiation was turned on at point A, fig C-10a, and off at point B, fig C-10c. Within 1 hour of initiation of the radiation, the cell again showed marked inhibition, as was observed during the first period of radiation. However, the increased inhibition lasted only about 10 minutes, after which the cell resumed normal activity. The cell did not again show signs of increased inhibition during the remaining 15 minutes of the radiation period, nor during the 70 minutes of post-radiation monitoring.

Only one cell which was classified as a responder to EM energy did not respond by an increase in inhibition. This cell was studied on 23 September 1980, and its activity is shown in fig C-11 through C-13. The cell's baseline activity was silent, with a few spikes, as shown in C-11). The cell was receiving synaptic input, as can be seen by the occasional inflections (excitatory) and deflections (inhibitory) in the cell's potential. Exposure to 135 mW/cm^2 began at 84 minutes into the experiment and ended at 176 minutes (point A, fig C-12d). A few minutes into radiation, the cell received large excitatory synaptic inputs (fig C-12a), some of which reached threshold, causing the cell to fire. Frequent, although irregular, firing began 68 minutes into exposure and ceased 24 minutes into post exposure. The cell then returned to a silent pattern for the remaining 25 minutes of the experiment (fig C-13).

Figures C-14 through C-16 show the inhibitory response of a VPN studied 27 January 1981. Baseline activity of this cell showed periodic increases and decreases in firing frequency, with occasional short periods of inhibition, as shown in fig C-14. The EM energy was turned on to a power density of 142 mW/cm^2 at 110 minutes into the experiment, and off at 205 minutes. Thirty minutes into the exposure period, the cell developed an increased number of inhibitory periods with increased duration, as seen in fig C-15. This inhibitory behavior diminished somewhat 20 minutes later, with only occasional periods of prolonged inhibition, as seen in fig C-16. No further inhibitions were observed in the last 70 minutes of the experiment.

The final cell showing a response was studied 29 January 1981, and is depicted in fig C-17 and C-18. This cell had a regular beating pattern, with only one brief inhibitory period through the 55 minutes of baseline. The microwave energy was turned on at 55 minutes, and 35 minutes later the cell developed an irregular beating pattern, as seen in fig C-17. The energy was turned off at 102 minutes. The cell continued its irregular pattern and became very inhibited starting at 5 minutes into post-exposure. The inhibitions continued for 20 minutes (fig C-18). The cell beat irregularly for 5 minutes, then regained a normal beating pattern for the remainder of the experiment.

POSSIBLE RESPONSE

Twenty-five exposed cells were classified as possibly showing an effect of the radiation. The experimental data from these cells did not show a clear normal/abnormal/normal pattern of response during the baseline/exposure/post-exposure periods, respectively. Eight cells classified as possible responders (22 May 1980, 4 June 1980, 10 June 1980, 14 July 1980, 22 July 1980, 24 July 1980, 16 September 1980, 22 January 1981) demonstrated increased inhibition during exposure, as determined by either a slow-down in firing frequency or an actual increase in the number and duration of inhibitory periods. However, this behavior did not cease when the radiation was turned off. The inhibitory behavior then could not be linked conclusively to the radiation.

Seven possible responding cells (5 June 1980, 2 September 1980, 23 December 1980, 3 February 1981, 4 February 1981, 9 March 1981, 14 April 1981) developed an increased activity during exposure. Four of these cells went from silent during baseline to beating during exposure; the other three simply developed an increased firing frequency during exposure. However, each cell's increased activity did not decrease during the post-exposure period, and again could not be attributed to the exposure.

Another seven of the cells in this category had several pattern changes throughout the experiment; therefore, a pattern change during exposure, even though a change occurred in post-exposure, could not be classified as a response. Table 2 summarizes the pattern changes of these cells, and in what periods the changes occurred. The boxed patterns are those which occurred during exposure. The patterns to the left of the box occurred during baseline, and to the right of the box during post-exposure.

Two cells (29 May 1980, 4 May 1981) showed no change in electrical behavior during exposure, but did develop inhibition during post-exposure. One cell (15 January 1981) exhibited a bursting pattern throughout the experiment, with the duration of the bursts increasing progressively. None of these three cells, then, could be classified as responding to the EM energy according to the predetermined criteria for responders, although the changing behavior in each cell may have been a response.

Date	Progression of Experiment
7-10-80	Irregular → regular beating → inhibited → regular beating
9-23-80	Silent → regular beating → increased frequency → decreased frequency → silent
1-15-81	Bursting → beating → bursting
1-22-81	Beating → inhibited → beating → inhibited
1-27-81	Short inhibitory periods → long inhibitory periods → short inhibitory periods → increased firing frequency → silent
3-02-81	Irregular → regular beating → bursting → beating → bursting
4-15-81	Silent → beating with increased frequency → steady beating → decreased frequency → steady beating → increased frequency → steady beating

Note: Boxed areas show firing pattern during exposure; baseline is shown to the left of box and post-exposure to the right of the box.

Table 2. Pattern changes in seven cells, possibly indicating a response.

3.3 CONTROL EXPERIMENTS EXAMINING THE EQUIPMENT

To verify that observed alterations in neuronal behavior were not caused by the equipment, several control experiments were run to look for potential equipment artifact. These experiments are summarized in table 3. Eleven experiments examined the electrical activity of the pipettes in seawater. In these tests, the procedure and equipment were identical to the nerve cell tests except that a nerve cell was not present. Of the six pipette experiments that utilized an exposure period, one performed 18 July 1980 did display sharp dc changes, as shown in fig 9a. During the pulsing (75 minutes) and baseline (70 minutes) periods, a gradual dc drop was observed, on the order of 60 μV per minute. The rate of this drop decreased in the first 85 minutes of exposure (156 mW/cm^2) to about 37.5 μV per minute. At 233 minutes, 88 minutes into exposure, a sharp increase in potential occurred, on the order of 25 mV in 5 minutes (point A, fig 9b). The potential continued to increase, at a slower rate of about 0.85 mV per minute (fig 9c). Another sharp dc change, a drop of 10 mV, occurred at 330 minutes, 18 minutes into post-exposure. The potential remained relatively stable, then suffered another sharp increase of 12 mV at 383 minutes (fig 9d). The recording period ended after a total of 6.5 hours with a net dc increase of 90 mV throughout the experiment. No sharp dc changes were observed in the other 10 pipette experiments except when the pipette came out of the seawater (5 December 1980, 10 December 1980, 29 December 1980).

On 29 December 1980, one of the pipettes being examined was used to determine the effects of the new gravity flow system on a shallowly placed pipette. Since the pipette came out of the water several times, this experiment was not considered a control for pipette artifact. Potential drift caused by either the amplifier or the recorder was examined on 27 October 1980. Very little drift occurred over a 23-hour testing period.

Date	Equipment Tested	Pulsing, min	Baseline, min	EM Exposure		Post-exposure, min	Total Time, min	Comments
				Power, mW/cm^2	Duration, min			
07-18-80	Pipette in seawater	75	70	154	165	80	390	Sharp and gradual drifts.
07-21-80	Pipette in seawater	20	90	154	150	260	520	No drift.
10-27-80	Amplifier and recorder	–	1380	–	–	–	1380	Gradual drift totalling 28 mV.
	Amplifier and recorder	–	1380	–	–	–	1380	Gradual drift totalling 22.5 mV.
12-05-80	Pipettes (2) in seawater	–	510	–	–	–	510	Noisy, used pump. Water level inconsistent. Slight dc drifts. No major changes except when water level dropped.
12-10-80	Pipettes (2) in seawater	–	440	–	–	–	440	Used pump. No major drifts except when water level dropped. At end of experiment, one pipette had higher resistance than at beginning.
12-29-80	Pipettes (2) in seawater	–	135	–	–	–	135	Gravity flow system. One pipette set deep, the other shallow. Deep one showed no large dc changes. Shallow one came out of water many times.
03-12-81	Pipettes (2) in seawater	–	65	123	50	10	125	No dc changes.
03-16-81	Pipettes (2) in seawater	–	50	123	30	10	90	No dc changes
	TOTAL	95	4120		395	360	4970	

Table 3. Control experiments examining potential equipment-caused artifacts.

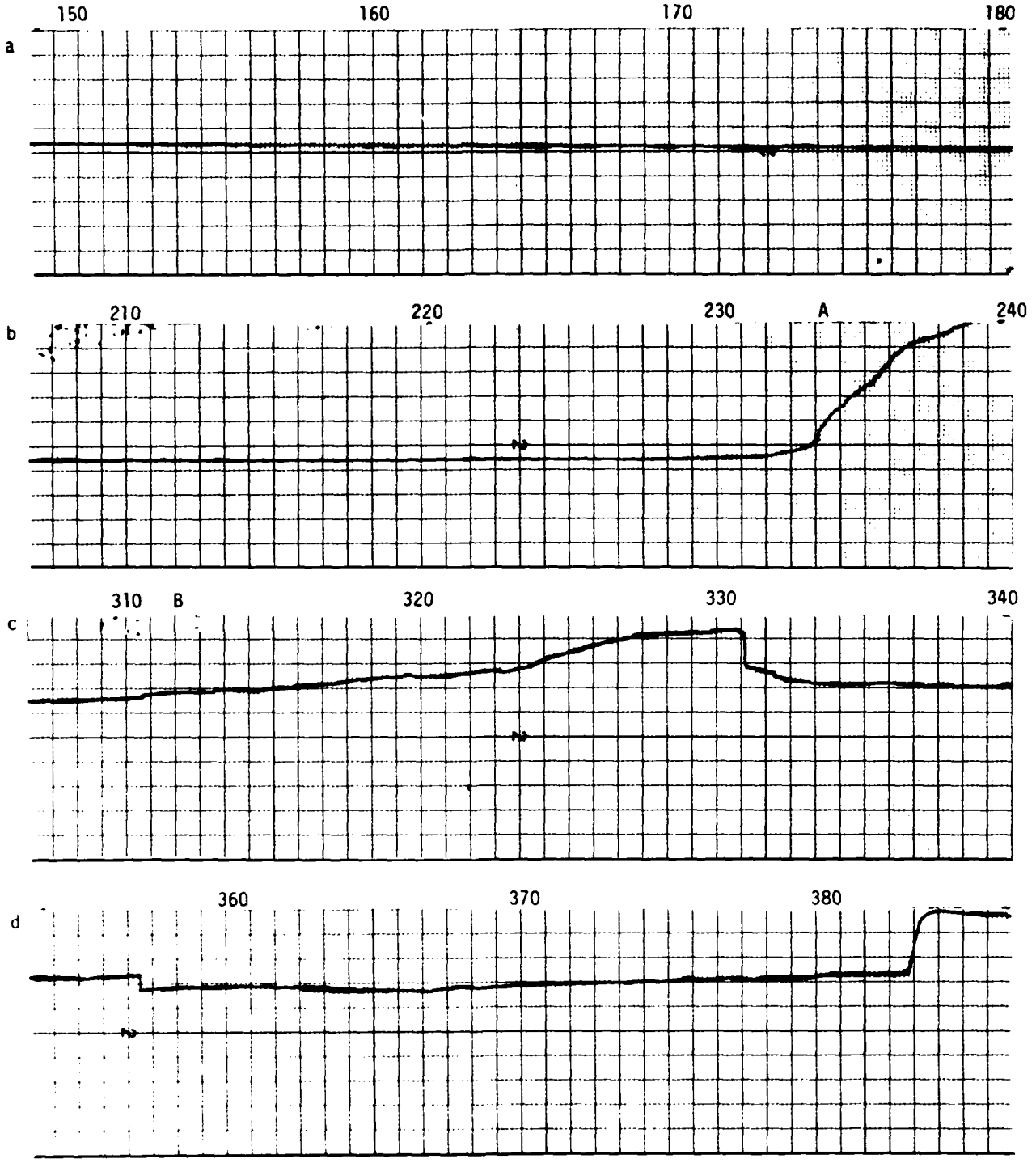

Figure 9. Pipette experiment, 18 July 1980.

4. DISCUSSION

4.1 POTENTIAL ARTIFACT FROM PIPETTES

One of the 11 pipette control tests showed sharp dc changes in the course of the experiment. Although these changes were not replicated in any of the other 10 tests, the occurrence of the sharp dc shifts may indicate the possibility of invalid results attributable to a pipette-caused artifact and should be explained. The most likely cause of the shifts is changes in tip potential. Micropipettes with small-diameter openings and filled with 3M KCl develop artifact tip potentials which are currently believed to be the result of wall charge effects (ref 16). As the resistance of the electrode (pipette) increases, the tip potential increases. A dc positioner is built into the amplifier to offset such potentials. The resistance of the pipette used on 18 July did increase over the 6.5 hours of study by approximately 5 MΩ. An increase in pipette resistance over time occurs occasionally, and is probably the result of the partial occlusion of the very small tip by a buildup of proteinaceous material or other debris. The observed potential shifts of the pipette in seawater on 18 July may have been due to pipette resistance changes. The lack of potential shifts observed in the other 10 pipette experiments indicates that the frequency of this phenomenon of pipette resistance change is low.

The question arises as to whether the possible dc shifting of a pipette may affect a cell's behavior. In a few of the experiments with cells, unexplained dc shifts did occur, and may have been the result of changing pipette tip potentials. Figure 10 shows the recording from a cell studied 18 August 1980. A sudden dc jump was observed at 28 minutes from initiation of the experiment (fig 10b). After 3.5 minutes, the experimenter brought the signal back on scale by adjusting the dc positioner of the amplifier. (The millivoltage needed was not recorded.) As can be seen, the behavior of the cell was not affected. The other cells observed to undergo such dc shifting likewise showed no change in behavior when the signal was repositioned.

4.2 NEURONAL RESPONSE TO EM ENERGY

Six cells were classified as responders to EM energy, representing 11% of the total cells exposed to EM energy (not including the cells classified as "U" for "unable to tell because of experimental problems"). Upon a similar analysis of the 15 control cells not exposed to EM energy, seven of the control cells [11 June 1980, 26 June 1980, 6 August 1980 (VPN), 18 August 1980, 17 March 1981 (VPN), 23 March 1981 (L10), 7 April 1981 (L10)] would have been classified as P for possible responders; seven cells [19 June 1980, 6 August 1980 (L10), 2 December 1980, 9 February 1981 (both), 17 March 1981 (L10), 7 April 1981 (X)] would have been classified N for no response, and one cell [23 March 1981 (L5)] would have been classified "U" for "unable to tell" because of experimental problems. Perhaps the six responders in the experimental group were erroneously classified as responders because of chance (ie, if each cell's change in behavior was not the result of EM exposure). Then 11% of the control group (excluding those classified as "U") should likewise have been classified as responders. However, no control cell was classified as responding. If the classification of responders is purely random, with an 11% chance of occurring regardless of exposure to microwave energy, the probability of finding no classified responders in 14 cells (the

16. Fein, H., An Introduction to Microelectrode Technique and Instrumentation, W-P Instruments, Inc, New Haven, CT, 1977.

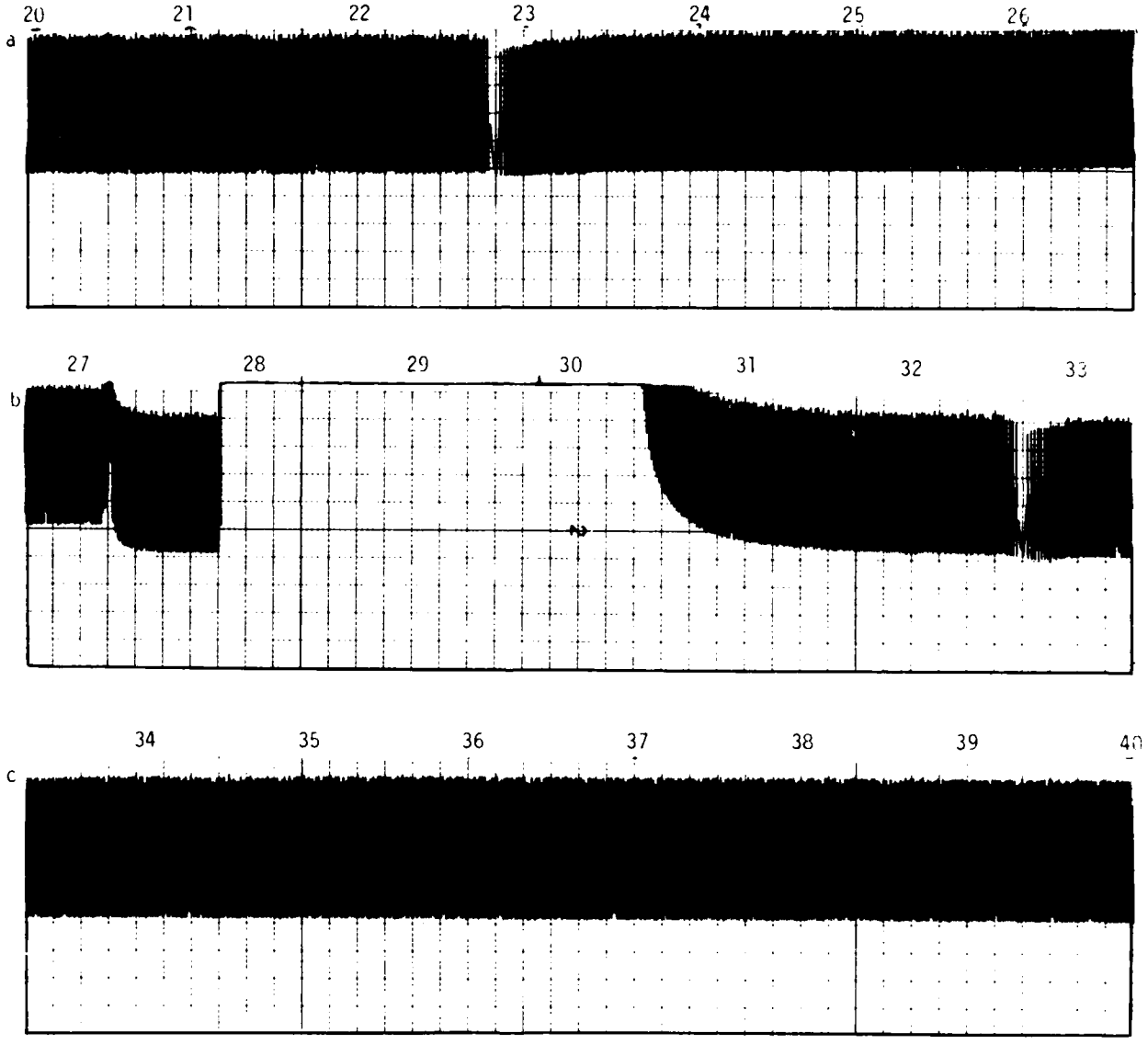

Figure 10. Response of cell studied 18 August 1980 to sudden dc shift.

control group less the one control cell classified as "U") is calculated to be less than 0.20. Although this probability is high, it still supports the conclusion that being classified as a responder is not random, and that at least some of the six experimental cells actually responded to the exposure.

Five of these responders developed increased inhibition. Possible causes of inhibition are

1. A change in the synaptic input, either an increase in inhibitory inputs or a decrease in excitatory inputs.
2. A change in membrane permeability either to increase potassium (K^+), calcium (Ca^{++}), or chloride (Cl^-) ion conductance, or to decrease sodium (Na^+) ion conductance.
3. A change in internal or external ionic concentrations.
4. An injection into the cell of hyperpolarizing dc current.

One of the responders developed more excitatory behavior, possibly caused by

1. A change in the synaptic input, either an increase in excitatory inputs or a decrease in inhibitory inputs.
2. A change in membrane permeability either to decrease K^+, Ca^{++}, or Cl^- conductance, or to increase Na^+ conductance.
3. A change in internal or external ionic concentrations.
4. An injection into the cell of depolarizing dc current.

A change in temperature is also a mechanism for inhibition or excitation in a neuron. However, temperature is not considered the mechanism in these experiments because of: (1) the temperature control procedures utilized in the experimental system; (2) the long-delayed onset of the EM energy responses, as opposed to the rapid onset of a temperature response; and (3) the observation that an increase in temperature usually causes an excitatory response, whereas the EM response in five out of six cells was inhibitory.

The reason for the excitatory response in one cell and the inhibitory responses in the others is unknown. One explanation is that the observed excitatory effect was indirectly caused by the microwave energy. Nerve cells commonly interact with each other, communicating inhibitory or excitatory signals. For example, nerve cell A may cause nerve cell B to be inhibited. If A is inhibited, its inhibitory signals to B will be decreased, and B will appear more excited. Perhaps the microwave exposure inhibited a cell like nerve cell A, and this response was indirectly observed as an increase in activity in a cell like nerve cell B, the cell being examined.

Neuronal response was also surprisingly inconsistent. This inconsistency may have resulted from the position of the cell being examined with respect to the incident field. Perhaps the responding cells were so positioned as to receive large dosages of energy (incident and reflected), and these large dosages are required before the cell is affected. However, as seen in table 1, the responding cells were exposed on the average to a lower power density than any of the other three classifications. If large power was required to elicit a response, then cells exposed to high power densities should have responded. However, they did not.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

A unique experimental system was developed to detect possible nonthermal effects of nonionizing EM energy on nerve cell behavior. Active nerve cells were maintained *in vitro* with microelectrodes inserted into the intracellular compartment, which allowed up to 10 hours of continuous recordings of each cell's electrical behavior. By means of this relatively long *in vitro* life period, the neurons were subjected to long periods of EM exposure to observe possible accumulative effects on neuronal activity. Thermal effects were eliminated by controlling the temperature of the exposed ganglion with a thermal electric cooling plate, and by constantly suffusing the cells with fresh, cooled seawater.

Of the 84 cells exposed, five showed inhibitory responses and one showed an excitatory response, which required from 6 to 82 minutes to develop. In each case, the cell returned to normal activity within 70 minutes of removing the EM field. One of the cells that showed an inhibitory response was further studied for its response to temperature changes. The cell responded to a 1°C increase in temperature by immediately increasing its firing frequency; hence the effect observed during radiation was not a thermal effect.

It appears from the data collected to date that interactions of EM energy on the electrical activity of nerve cells does exist, and that this interaction is not heat-related. Understanding the mechanism of this interaction will provide a valuable tool in determining the hazards of nonionizing EM energy and the necessary safety limits of exposure.

5.2 RECOMMENDATIONS

POWER LEVELS

High power densities (up to 310 mW/cm^2) were used in these experiments because of an initial assumption that exposure to high power levels, while maintaining thermal control, would yield a high frequency of nonthermal response. However, the cells that did show a response were exposed to power levels lower than the average level used in these experiments. Other research (ref 17, 18) utilizing MHz frequencies amplitude modulated to less than 20 Hz observed the presence of power density windows (a narrow range of power densities at which effects most often occur). These windows occurred at low power densities (less than 1 mW/cm^2), which indicated that exposure to high power levels will not increase the frequency of response. Further research utilizing the experimental system described in this report should examine the possibility that effects may be more frequently observed at power densities lower than those used in these experiments.

ANALYSIS METHOD

Examination of the recordings from the 15 cells used for controls indicates that most of the cells do not maintain a regular electrical firing pattern throughout the experiment,

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17. Blackman, C.F., et al, Induction of Calcium-Ion Efflux from Brain Tissue by Radio-Frequency Radiation: Effects of Modulation Frequency and Field Strength, Radio Science, 14(6S):93, 1979.

18. Blackman, C.F., et al, Induction of Calcium-Ion Efflux from Brain Tissue by Radiofrequency Radiation: Effect of Sample Number and Modulation Frequency on the Power-Density Window, Bioelectromagnetics, 1(1):35, 1980.

which makes the identification of a response difficult. In the foregoing sections, a response was claimed only in the most obvious cases of normal/abnormal/normal behavior coinciding appropriately with the baseline/exposure/post-exposure periods. This criterion, however, assumes that the responding cell will recover in post-exposure, which is probably not a valid assumption, and many responding cells may have been ignored. A more stringent analysis method needs to be employed, either to compare control versus experimental firing patterns by means of computer statistical analysis, or to examine a less time-variable aspect of the neuronal electrical activity, such as membrane ionic conductances.

Although monitoring ionic conductances limits the experiment to the observation of only one possible mechanism of the response, membrane permeability change is a very probable mechanism. Several researchers have suggested altered membrane permeability as a possible mechanism of the EM effects (ref 11, 17–20). Microwave-induced changes in membrane ionic conductances can be directly measured by using voltage clamp (VC) techniques (ref 21–23). In VC, the current needed to maintain a constant voltage across the membrane is measured and represents the membrane current, which is a summation of all the ionic currents. Each individual ion's contribution to the total current can be determined by: (1) blocking ion channels with specific drugs and altering the external ionic environment around the cell while voltage clamping; and (2) clamping to different voltages, measuring the reversal potential (E_r, the potential at which current flow changes direction), and relating E_r to each specific ion's equilibrium potential (ref 24–25). Knowing the ionic currents, the ionic conductances can be calculated.

J. L. Schwartz used VC to observe possible magnetic field-induced changes on membrane potentials and transmembrane currents in the lobster (ref 26), but he had numerous experimental difficulties. His recommendations for future work with this approach included: (1) using a more resistant nerve cell preparation which would survive more than an hour; and (2) voltage clamping with intracellular electrodes instead of the extracellular method he used (personal correspondence, 1978). The experimental system described in this report is already operational with intracellular electrodes, and with a nerve cell preparation that survives many hours of experimentation. Thus monitoring ionic conductance changes by using VC is a logical continuation of the present study.

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19. Pickard, W.F., and F.J. Rosenbaum, Biological Effects of Microwaves at the Membrane Level: Two Possible Athermal Electrophysiological Mechanisms and a Proposed Experimental Test, Mathematical Biosciences, 39:235, 1978.

20. Barnes, F.S., and C.L. Hu, Model for Some Nonthermal Effects of Radio and Microwave Fields on Biological Membranes, IEEE Trans MTT, 25:742, 1977.

21. Smith, T.G., et al, Voltage Clamping with Microelectrodes, J of Neuroscience Methods, 3:105, 1980.

22. Wilson, W.A., and M.M. Goldner, Voltage Clamping with a Single Microelectrode, J of Neurobiology, 6:411, 1975.

23. Frank, K., and I. Tauc, Voltage-Clamp Studies of Molluscan Membrane Properties, in Cellular Functions of Membrane Transport, J.F. Hoffman (ed), 1964.

24. Katz, B., Nerve, Muscle, and Synapse, McGraw-Hill Book Co, New York, NY, 1966.

25. Hodgkin, A.L., and A.F. Huxley, Currents Carried by Sodium and Potassium Ions through the Membrane of the Giant Axon of *Loligo*, J Physiol, 116:449, 1952.

26. Schwartz, J.L., Influence of a Constant Magnetic Field on Nervous Tissue. II. Voltage-Clamp Studies, IEEE Trans on Biomed Eng, 26:238, 1979.

REFERENCES

1. Justesen, D.R., Diathermy versus the Microwaves and Other Radio Frequency Radiation: A Rose by Another Name is a Cabbage, Radio Science 12(3):355, 1977.

2. Zaret, M.M., et al, Clinical Microwave Cataracts, in Biological Effects and Health Implications of Microwave Radiation, Symposium Proceedings, S.F. Cleary (ed), US Dept of Health, Education and Welfare, 1970.

3. Baranski, S., and P. Czerski, Biological Effects of Microwaves, Dowden, Hutchinson, and Ross, Inc, Stroudsburg, PA, 1976.

4. Takashima, S., B. Onaral, and H.P. Schwan, Effects of Modulated RF Energy on the EEG of Mammalian Brains. II. Appearance of Fast and Slow Waves After Chronic Irradiation, in Symposium on Electromagnetic Fields in Biological Systems, S.S. Stuchly (ed), International Microwave Power Institute, Edmonton, Alberta, 1979.

5. Servantie, B., A.M. Servantie, and J. Etienne, Synchronization of Cortical Neurons by a Pulsed Microwave Field as Evidenced by Spectral Analysis of Electrocorticograms from the White Rat, Annals of New York Acad of Sciences, 247:82, 1975.

6. Bawin, S.M., et al, Reinforcement of Transient Brain Rhythms by Amplitude-Modulated VHF Fields, in Biologic and Clinical Effects of Low Frequency Magnetic and Electrical Fields, J.G. Llaruado, et al (eds), Charles C. Thomas, Springfield, IL, 1974.

7. Albert, E., and M. DeSantis, Do Microwaves Alter the Nervous System Structure? New York Acad of Sciences, 247:87, 1975.

8. Albert, E., and M. DeSantis, Histological Observations on Central Nervous System, HEW (FDA) 77-8010, 11:299, 1976.

9. Frey, A.H., Behavioral Biophysics, Psych Bull, 63:322, 1965.

10. Wachtel, H., et al, Effects of Low-Intensity Microwaves on Isolated Neurons, New York Acad of Sciences, 247:46, 1975.

11. Seaman, R.L., and H. Wachtel, Slow and Rapid Responses to CW and Pulsed Microwave Radiation by Individual *Aplysia* Pacemakers, J of Microwave Power, 13(1):77, 1978.

12. Frazier, W.T., et al, Morphology and Functional Properties of Identified Neurons in the Abdominal Ganglion of *Aplysia californica*, J Neurophys, 30:1288, 1967.

13. Andresen, M.D., and A.M. Brown, Photoresponses of a Sensitive Extraretinal Photoreceptor in *Aplysia*, J of Physiol (London), 287:267, 1979.

14. Brown, A.M., and H.M. Brown, Light Response of a Giant *Aplysia* Neuron, J Gen Physiol, 62:239, 1973.

15. Kandel, E.R., Cellular Basis of Behavior, W.H. Freeman and Co, San Francisco, CA, 1976.

16. Fein, H., An Introduction to Microelectrode Technique and Instrumentation, W-P Instruments, Inc, New Haven, CT, 1977.

17. Blackman, C.F., et al, Induction of Calcium-Ion Efflux from Brain Tissue by Radio-Frequency Radiation: Effects of Modulation Frequency and Field Strength, Radio Science, 14(6S):93, 1979.

18. Blackman, C.F., et al, Induction of Calcium-Ion Efflux from Brain Tissue by Radio-frequency Radiation: Effect of Sample Number and Modulation Frequency on the Power-Density Window, Bioelectromagnetics, 1(1):35, 1980.

19. Pickard, W.F., and F.J. Rosenbaum, Biological Effects of Microwaves at the Membrane Level: Two Possible Athermal Electrophysiological Mechanisms and a Proposed Experimental Test, Mathematical Biosciences, 39:235, 1978.

20. Barnes, F.S., and C.L. Hu. Model for Some Nonthermal Effects of Radio and Microwave Fields on Biological Membranes, IEEE Trans MTT, 25:742, 1977.

21. Smith, T.G., et al, Voltage Clamping with Microelectrodes, J of Neuroscience Methods, 3:105, 1980.

22. Wilson, W.A., and M.M. Goldner, Voltage Clamping with a Single Microelectrode, J of Neurobiology, 6:411, 1975.

23. Frank, K., and I. Tauc, Voltage-Clamp Studies of Molluscan Membrane Properties, in Cellular Functions of Membrane Transport, J.F. Hoffman (ed), 1964.

24. Katz, B., Nerve, Muscle, and Synapse, McGraw-Hill Book Co, New York, NY, 1966.

25. Hodgkin, A.L., and A.F. Huxley, Currents Carried by Sodium and Potassium Ions through the Membrane of the Giant Axon of *Loligo*, J Physiol, 116:449, 1952.

26. Schwartz, J.L., Influence of a Constant Magnetic Field on Nervous Tissue. II. Voltage-Clamp Studies, IEEE Trans on Biomed Eng, 26:238, 1979.

APPENDIX A. CELLS NOT EXPOSED TO EM ENERGY

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Date	Cell	Type	Pulsing, min	Baseline, min	Total Time, min	Comments
04-02-80	R15	Bursting	–	150	150	No cooling plate. Cell came out of water in middle of experiment. Water was added. No change in cell's behavior throughout experiment.
04-02-80	L10	Beating	–	120	120	Cell's beating frequency increased in first half of experiment. Cell came out of fluid and water was added. Increased firing frequency towards end of experiment.
04-10-80	R15	Bursting	–	45	45	No change in cell's behavior.
04-14-80	VPN	Beating	–	90	90	Noise.
04-14-80	R15	Bursting	–	45	45	Noise.
04-22-80	L10	Silent	–	60	60	Noise.
05-05-80	VPN	Beating	–	120	120	Noise.
05-12-80	R15	Bursting	–	20	20	Noise.
06-04-80	X	Beating	–	20	20	Developed bursting pattern 5 minutes into experiment.
06-11-80	VPN	Beating	–	360	360	CONTROL. Increased sensitivity to light over time. Became irregular after 4 hours. Pipette came out at 300 minutes and was reinserted.
06-19-80	VPN	Beating	–	305	305	CONTROL. Increased inhibition over time.
06-25-80	X	Beating	115	–	115	Cell damaged.
06-26-80	VPN	Beating	45	230	275	CONTROL. Strongly inhibited first 2 hours. Pipette came out of cell at 275 minutes. Some noise.
	X	Beating	30	60	90	Some dc shifting.
06-27-80	VPN	Beating	20	145	165	Added curare.
07-03-80	X	Beating	30	–	30	Cell damaged.
07-09-80	VPN	Beating	35	20	55	Pipette came out.
08-06-80	L10	Beating	–	217	217	CONTROL. Regular pattern throughout experiment.
08-06-80	VPN	Beating	–	410	410	CONTROL. Became irregular at 90 minutes. Then was silent for 180 minutes. Pipette came out at 285 minutes and was reinserted.
08-11-80	X	Bursting	–	80	80	Went from silent to regular bursting pattern.
08-11-80	X	Bursting	–	80	80	Went from beating to bursting pattern.
08-12-80	X	Silent	20	30	50	Silent and irregular. Cell damaged.
08-12-80	X	Beating	40	75	115	Went irregular at 30 minutes into baseline. Pipette came out for 70 minutes.
08-18-80	X	Beating	42	225	267	CONTROL. Pipette came out at 30 minutes. Cell became irregular at 150 minutes.
08-27-80	X	Beating	30	40	70	Pipette came out at 70 minutes.
08-27-80	X	Bursting	30	40	70	Pipette came out at 70 minutes.
08-28-80	VPN	Beating	20	210	230	Noisy, used pump.

Date	Cell	Type	Pulsing, min	Baseline, min	Total Time, min	Comments
08-28-80	VPN	Beating	30	320	350	Noisy, used pump.
12-02-80	VPN	Beating	–	160	160	CONTROL. Firing frequency decreased throughout day.
12-31-80	X	Beating	–	210	210	Noisy, used pump.
12-31-80	X	Beating	–	80	80	Noisy, used pump.
01-02-81	X	Bursting	–	115	115	Noisy, used pump.
02-04-81	X	Beating	–	70	70	Cell damaged.
02-09-81	X	Beating	–	115	115	CONTROL. Cell developed regular pattern 55 minutes into baseline. Continued throughout experiment.
02-09-81	X	Beating	–	105	105	CONTROL. Cell developed regular pattern 65 minutes into baseline. Continued throughout experiment.
02-23-81	X	Beating	20	25	45	Some periodic hyperpolarizations. Otherwise regular beating pattern.
03-17-81	VPN	Beating	30	335	385	CONTROL. Gradual increase in firing frequency for first 210 minutes, then became increasingly irregular. Cell stopped firing at 300 minutes.
3-17-81	L10	Beating	35	250	285	CONTROL. Gradual increase in firing frequency throughout experiment. Pipette came out at end of experiment.
03-23-81	L10	Beating	15	400	415	CONTROL. Cell was silent with a lot of synaptic input (including deep hyperpolarizations) the first 15 minutes of baseline and again for 15 minutes starting 60 minutes into baseline. The cell then developed a regular beating pattern with occasional hyperpolarizations. The beating frequency increased over time. Went into bursting pattern 2 hours into baseline, then returned to regular fast beating pattern after 15 minutes.
03-23-81	L5	Beating	10	330	340	CONTROL. Cell developed slightly irregular beating pattern 1 hour into baseline lasting until 3 hours 10 minutes into baseline. Cell beat more regularly through rest of experiment.
04-07-81	L10	Bursting	–	205	205	CONTROL. Cell was silent with occasional hyperpolarizations until 2 hours into baseline. The beating frequency then gradually increased over time.
04-07-81	X	Silent	15	180	195	CONTROL. Cell started with beating pattern. Went silent 30 minutes into baseline with occasional spikes. Remained silent through rest of experiment.
	TOTAL		612	6117	6729	
	AVERAGE		32 (n=19)	153 (n=40)	160 (n=42)	

APPENDIX B. CELLS EXPOSED TO EM ENERGY

Date	Cell	Type	Pulsing, min	Baseline, min	EM Exposure		Post Exposure, min	Total Time, min	Classification	Comments
					Power, mW/cm^2	Duration, min				
04-15-80	R15	Bursting	–	30	12	5	7	177	U	Electrical noise
			–	–	31	5	90	–		
			–	–	154	15	25	–		
05-07-80	X	Beating	–	20	126	6	31	250	R	Response was complete inhibition.
			–	–	126	12	181	–		Recovered in post-exposure.
05-15-80	R15	Bursting	–	35	106	9	–	44	N	No observable effect.
05-20-80	VPN	Beating	–	37	126	25	32	196	N	No observable effect.
			–	–	154	78	24	–		
05-22-80	VPN	Beating	–	100	142	20	20	140	U	Irregular in baseline.
05-22-80	VPN	Beating	–	40	126	25	50	115	P	Increased inhibition during radiation. Decreased to normal in post-exposure for a short time; 50 minutes into post-exposure, became completely inhibited.
05-27-80	VPN	Beating	–	80	142	23	40	255	N	No observable effect.
			–	–	157	65	47	–		
05-29-80	VPN	Beating	–	125	154	130	120	405	P	Irregular in post-exposure.
			–	–	131	30	–	–		
06-04-80	VPN	Beating	–	80	169	120	65	265	P	Temporarily inhibited during radiation.
06-05-80	VPN	Beating	–	110	178	60	110	325	P	Increased firing frequency during second radiation.
			–	–	148	45	–	–		
06-10-80	VPN	Beating	–	65	174	335	75	475	P	Long inhibition not related to light occurred during radiation. Some recovery during post-exposure.
06-12-80	VPN	Beating	–	50	185	55	–	105	U	Cell damaged.
06-18-80	VPN	Beating	–	70	188	90	133	293	R	Increased inhibition during radiation. Decreased inhibition in post-exposure.
07-02-80	X	Beating	35	30	177	65	170	300	U	Used curare.
07-03-80	VPN	Beating	40	110	160	90	35	275	U	Cell damaged.

Date	Cell	Type	Pulsing, min	Baseline, min	EM Exposure		Post Exposure, min	Total Time, min	Classification	Comments
					Power, mW/cm^2	Duration, min				
07-08-80	VPN	Beating	15	225	162	70	30	340	N	No observable effect.
07-10-80	VPN	Beating	45	75	162	185	75	380	P	Cell irregular during baseline. Became regular during radiation. Inhibited at end of radiation. Recovered in post-exposure.
07-14-80	VPN	Beating	45	125	172	265	65	500	P	Slight inhibition during radiation. Did not recover in post-exposure.
07-16-80	X	Beating	85	50	158	140	60	335	N	No observable effect.
07-17-80	X	Beating	20	75	111	65	180	500	R	Became inhibited 30 minutes after initiation of first radiation. Completely recovered 30 minutes after EM energy turned off.
			–	–	31	90	70	–		
07-22-80	VPN	Beating	35	108	92	84	30	257	P	Frequent inhibitions during radiation. Did not recover in post-exposure.
07-23-80	X	Beating	45	90	92	120	145	400	U	Noise.
07-24-80	X	Beating	40	110	92	180	210	540	P	Frequent inhibitions during radiation. Did not recover in post-exposure.
07-28-80	X	Beating	–	70	92	40	–	110	U	Pipette came out during radiation.
07-29-80	X	Beating	60	90	92	135	120	405	N	No observable effect.
08-04-80	VPN	Beating	–	127	172	80	–	327	U	Problems with pump.
			–	–	168	60	60	–		
08-21-80	X	Bursting	–	–	148	120	40	160	U	No baseline data.
08-21-80	X	Silent	–	–	148	120	40	160	U	Became irregular after radiation, but no baseline data.
09-02-80	X	Silent	45	50	160	130	165	390	N	No observable effect.
09-02-80	X	Beating	45	40	160	130	180	395	P	Firing frequency increased during radiation. Did not decrease in post-exposure.
09-08-80	X	Beating	10	85	175	120	90	305	U	Noisy, used pump.

Date	Cell	Type	Pulsing, min	Baseline, min	EM Exposure		Post Exposure, min	Total Time, min	Classification	Comments
					Power, mW/cm^2	Duration, min				
09-08-80	VPN	Beating	30	75	175	120	90	315	N	Noisy, used pump, but no observable effect.
09-09-80	L10	Beating	35	210	151	60	40	345	U	Paper speed too slow.
09-09-80	VPN	Beating	40	210	151	60	40	350	U	Pipette came out during exposure, cell never recovered.
09-16-80	VPN	Beating	–	185	154	120	65	370	P	Decrease in firing frequency during exposure. No recovery in post-exposure.
09-16-80	VPN	Beating	–	145	154	120	65	330	N	No observable effect. However, went into bursting pattern in post-exposure, probably because water level too low (pump was used).
09-23-80	R14	Beating	–	80	135	95	45	220	P	Cell mostly silent through baseline. Started regular beating pattern just before end of baseline. Frequency of beating increased first 50 minutes of exposure, then frequency started to decrease. Cell went silent 5 minutes into post-exposure.
09-23-80	R2	Silent	–	85	135	90	45	220	R	Mostly silent until 80 minutes into exposure. Went silent again 25 minutes into post-exposure.
12-11-80	X	Beating	–	60	92	85	10	155	U	Noisy, used pump.
12-23-80	VPN	Beating	–	125	125	85	25	235	U	Cell looked damaged in baseline. Was beating regularly, then became irregular, then quit, then started bursting. Became regular again 10 minutes into exposure. Continued regular beating through rest of experiment.
12-23-80	L10	Silent	–	105	125	85	25	215	P	Silent with a lot of synaptic input in baseline. Started beating 60 minutes into exposure. Continued through post-exposure.

Date	Cell	Type	Pulsing, min	Baseline, min	EM Exposure		Post Exposure, min	Total Time, min	Classification	Comments
					Power, mW/cm^2	Duration, min				
01-14-81	L6	Bursting	–	225	92	70	–	295	U	Noisy, used pump. Pipette came out twice during exposure.
01-14-81	R15	Beating	–	135	92	70	10	215	U	Noisy, used pump. Increasing the water flow during exposure caused an immediate increase in firing frequency.
01-15-81	R11	Bursting	–	130	92	120	120	370	P	Used gravity flow system. Bursting pattern for first hour of baseline, then developed beating pattern. Went back to bursting pattern 40 minutes into exposure. Continued through post-exposure. Pipette came out at end of exposure and was reinserted.
01-15-81	L5	Bursting	–	105	92	120	160	385	P	Bursts got progressively longer in duration throughout experiment.
01-22-81	L5	Beating	–	90	92	60	50	200	P	Firing frequency decreased throughout experiment.
01-22-81	R16	Beating	–	110	92	60	50	220	P	Became inhibited before exposure, recovered slightly during exposure, then became very inhibited during exposure. Did not recover in post-exposure.
01-27-81	L10	Beating	50	85	142	95	95	325	P	Had periodic inhibitory periods in baseline. Duration of these periods increased 10 minutes into exposure. Cell went back to normal 20 minutes into exposure. Firing frequency increased 35 minutes into exposure. Cell looked damaged 65 minutes into exposure (unknown reason). Stopped beating 10 minutes after exposure. Did not recover.

Date	Cell	Type	Pulsing, min	Baseline, min	EM Exposure		Post Exposure, min	Total Time, min	Classification	Comments
					Power, mW/cm^2	Duration, min				
01-27-81	VPN	Beating	30	80	142	95	95	300	R	Number of inhibitory periods increased 30 minutes into exposure. Recovered 45 minutes into exposure. No inhibitory periods after 15 minutes post-exposure.
01-28-81	R15	Bursting	–	110	92	120	60	290	U	Pipette came out twice during baseline and once during exposure. Last reinsertion damaged cell. Interburst intervals seemed to increase during exposure.
01-28-81	L11	Beating	–	70	92	120	60	250	U	Pipette came out twice during exposure. Paper speed too slow to analyze spike frequency.
01-29-81	X	Beating	–	110	92	70	–	180	U	Slow beater. Pipette came out twice during exposure. Cell became damaged on second reinsertion. Long inhibitory period (40 minutes) occurred 25 minutes into exposure.
01-29-81	X	Bursting	–	110	92	70	15	195	N	Pipette came out during exposure but cell not damaged. Tapping on other pipette damaged this cell for entire post-exposure period. No observable effect during exposure.
01-29-81	X	Beating	–	55	92	45	80	180	R	Became irregular 40 minutes into exposure. Became inhibited 5 minutes after exposure. Went back to irregular pattern 25 minutes into post-exposure. Became regular 30 minutes into post-exposure.
01-30-81	X	Beating	–	85	154	20	85	350	U	Cell damaged.
			–	–	138	30	130	–		

Date	Cell	Type	Pulsing, min	Baseline, min	EM Exposure		Post Exposure, min	Total Time, min	Classification	Comments
					Power, mW/cm^2	Duration, min				
02-03-81	L10	Beating	30	160	148	120	85	395	P	Was inhibited for 10 minutes during baseline. Gradually speeded up in firing frequency during exposure. Continued through post-exposure. Pipette came out 85 minutes into post-exposure.
02-03-81	VPN	Beating	30	160	148	120	5	530	U	More inhibitory periods 95 minutes into exposure. Pipette came out at end of exposure period. Pulsed for 25 minutes at beginning of post-exposure period. No inhibitory periods in post-exposure.
			25	–	–	–	190	–		
02-04-81	L7	Beating	–	45	146	25	15	85	U	Noisy. Cell became irregular and inhibited during baseline. Seemed to become more regular 15 minutes into exposure. Became damaged 15 minutes into post-exposure.
02-04-81	X	Beating	–	70	146	60	90	220	N	No observable effects.
02-04-81	L11	Beating	–	10	145	70	60	200	P	Firing frequency slowed during first exposure, then speeded up. Firing frequency continued to increase through first post-exposure and through second exposure.
			–	–	92	50	10	–		
02-05-81	X	Beating	–	60	154	40	–	100	U	Noisy, pipette came out.
02-05-81	X	Beating	–	70	154	45	80	310	U	Noisy.
			–	–	154	75	45	–		
02-05-81	X	Beating	–	10	154	75	40	125	U	Noisy.
02-05-81	X	Beating	–	50	123	90	15	155	U	Noisy. Cell beating became very regular during exposure. Stayed regular through post-exposure.
02-10-81	X	Beating	–	20	120	30	–	215	U	Became slightly irregular during exposure and post-exposure. Baseline not long enough.
			–	–	146	70	95	–		
02-10-81	X	Bursting	10	5	120	30	–	210	U	Baseline not long enough.
			–	–	146	70	95	–		

Date	Cell	Type	Pulsing, min	Baseline, min	EM Exposure		Post Exposure, min	Total Time, min	Classification	Comments
					Power, mW/cm^2	Duration, min				
02-24-81	R15	Bursting	10	35	112	45	40	130	N	No observable effect.
03-02-81	L10	Beating	20	195	92	100	100	415	P	Developed regular beating pattern 40 minutes into baseline. Became bursting 40 minutes into exposure. Stopped bursting 60 minutes into exposure and increased its firing frequency. Became bursting again 15 minutes into post-exposure. Firing frequency stayed fast.
03-02-81	VPN	Beating	20	195	92	100	100	415	N	No observable effect.
03-03-81	L10	Beating	20	130	114	145	160	455	N	No observable effect.
03-03-81	VPN	Beating	15	105	114	145	160	425	N	No observable effect.
03-09-81	L10	Beating	40	130	134	60	150	380	P	Inhibited for 15 minutes during baseline. Slowly increased firing frequency during exposure. Stayed fast in post-exposure.
03-09-81	X	Beating	10	130	134	60	150	350	N	No observable effect.
03-14-81	VPN	Beating	30	180	308	120	100	430	N	Used 2 GHz. Cell developed regular beating pattern 110 minutes into baseline. Beating frequency increased gradually throughout experiment with occasional hyperpolarizations and inhibitions. No observable response to exposure.
04-14-81	L10	Beating	30	180	308	120	100	430	P	Cell was beating for first 10 minutes of baseline, then became silent with occasional spikes. Gradually started beating 80 minutes into exposure. Continued regular beating (no acceleration) till end of experiment.
04-15-81	L10	Beating	10 25	75 40	– 308	– 35	– 40	225 –	N	Pipette came out so cell had to be pulsed again after reinsertion. Beating frequency continually slowed down throughout experiment. No observable response to exposure.

Date	Cell	Type	Pulsing, min	Baseline, min	EM Exposure		Post Exposure, min	Total Time, min	Classification	Comments
					Power, mW/cm^2	Duration, min				
04-15-81	R11	Beating	10	145	308	35	40	230	P	Cell was silent first 20 minutes of baseline. Started beating with increasing frequency, in bursting patterns. Frequency stabilized (stopped increasing) at initiation of exposure, although still continued bursting pattern. Frequency slowed 15 minutes into exposure, then returned to normal after 5 minutes. Frequency increased 5 minutes into post-exposure, then returned to normal after 10 minutes.
04-20-81	VPN	Beating	20	160	302	110	130	420	N	Cell had regular beating until 110 minutes into baseline, then developed irregular pattern which continued through rest of experiment. Did temperature studies in post-exposure. An increase in temperature resulted in increased firing frequency. No observable response to exposure.
04-20-81	VPN	Beating	30	160	302	110	130	430	N	Cell beat regularly throughout experiment with intermittent bursting. Did temperature studies in post-exposure. An increase in temperature caused an increase in firing frequency. No observable response to exposure.
04-23-81	X	Beating	30	140	298 298	65 20	85 10	350	N	Cell gradually increased its firing frequency over time. No observable response to exposure.
04-30-81	X	Bursting	10	160	308	225	30	425	N	No observable effect.
04-30-81	X	Beating	25	115	308	225	30	395	N	Cell beat regularly. Went into slightly irregular pattern 25 minutes into baseline. Returned to regular pattern 105 minutes into baseline. No observable response to exposure.

Date	Cell	Type	Pulsing, min	Baseline, min	EM Exposure		Post Exposure, min	Total Time, min	Classification	Comments
					Power, mW/cm^2	Duration, min				
05-04-81	VPN	Beating	10	140	308	220	115	485	U	Cell was very irregular throughout experiment.
05-04-81	X	Beating	25	65	308	220	60	370	P	No change in beating pattern throughout exposure period. Firing frequency slowed when energy was turned off and remained slow first 10 minutes of post-exposure. Frequency also slowed when temperature was either increased or decreased.
TOTALS	84		1230	8222		8662	6830	24944		
AVERAGE			31 (n=40)	98		103	81	297		

APPENDIX C. NEUROELECTRICAL ACTIVITY OF NEURONS RESPONDING TO EM EXPOSURE

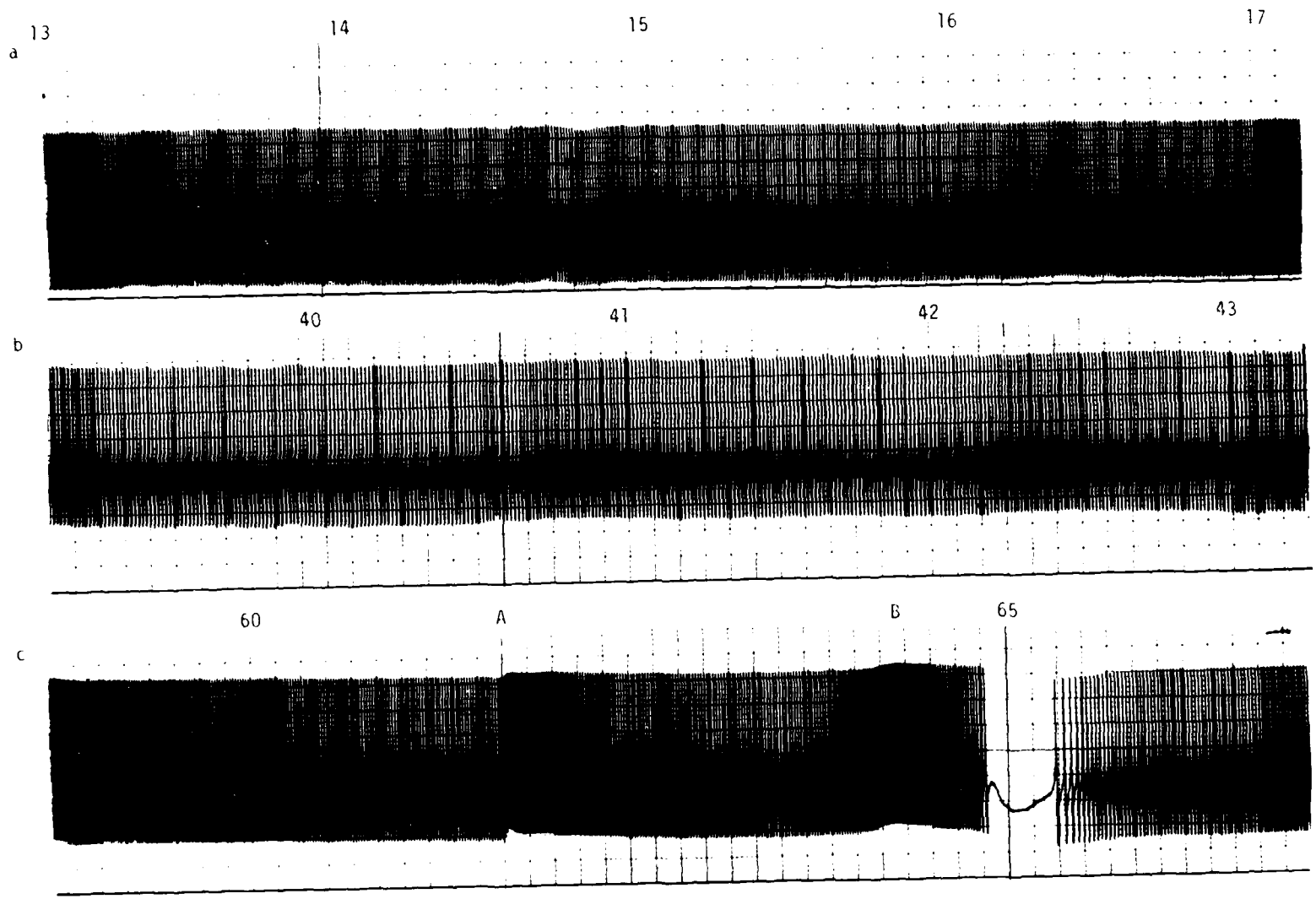


Figure C-1a-c. Baseline period of cell studied 18 June 1980.

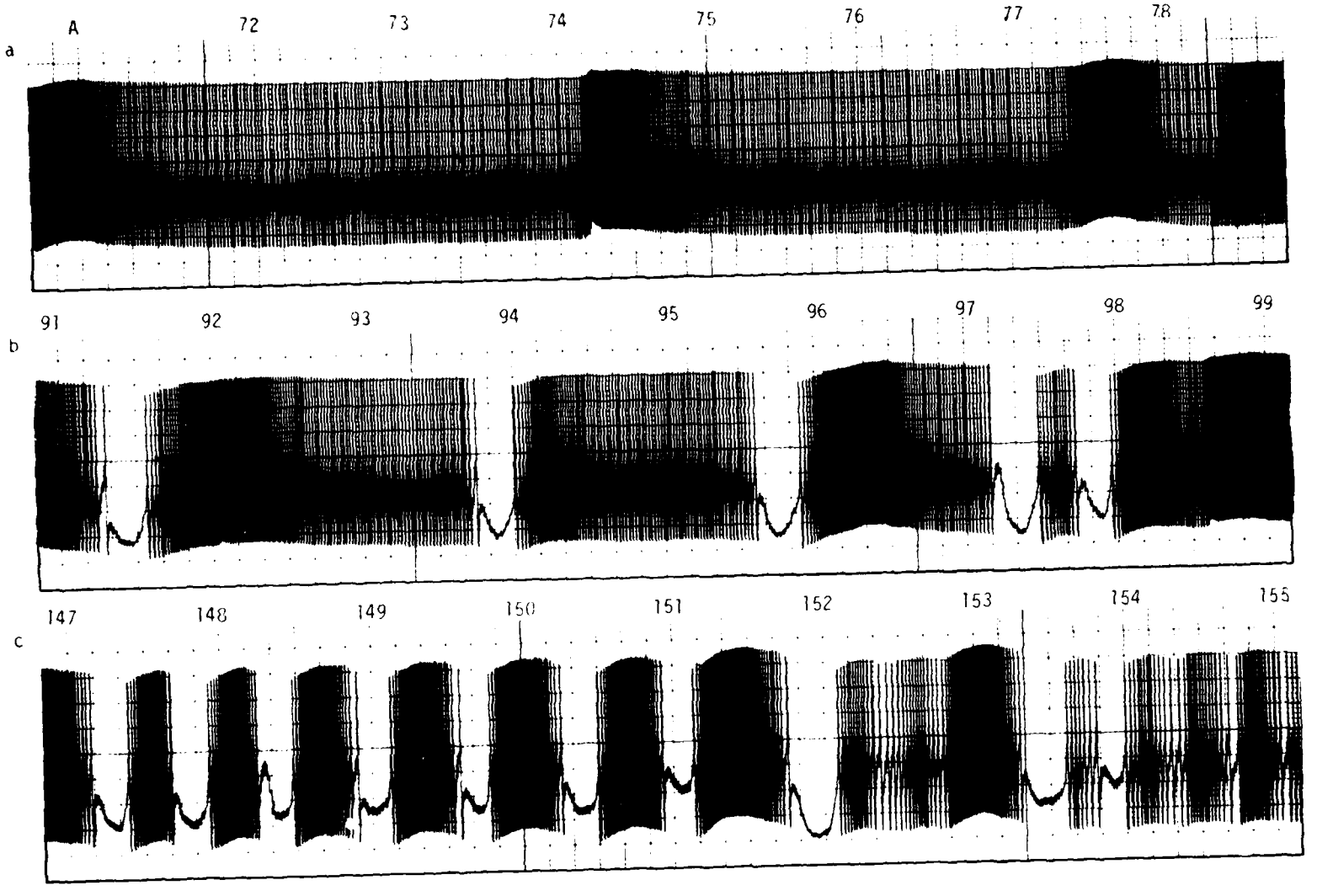


Figure C-2a-c. Exposure period of cell studied 18 June 1980.

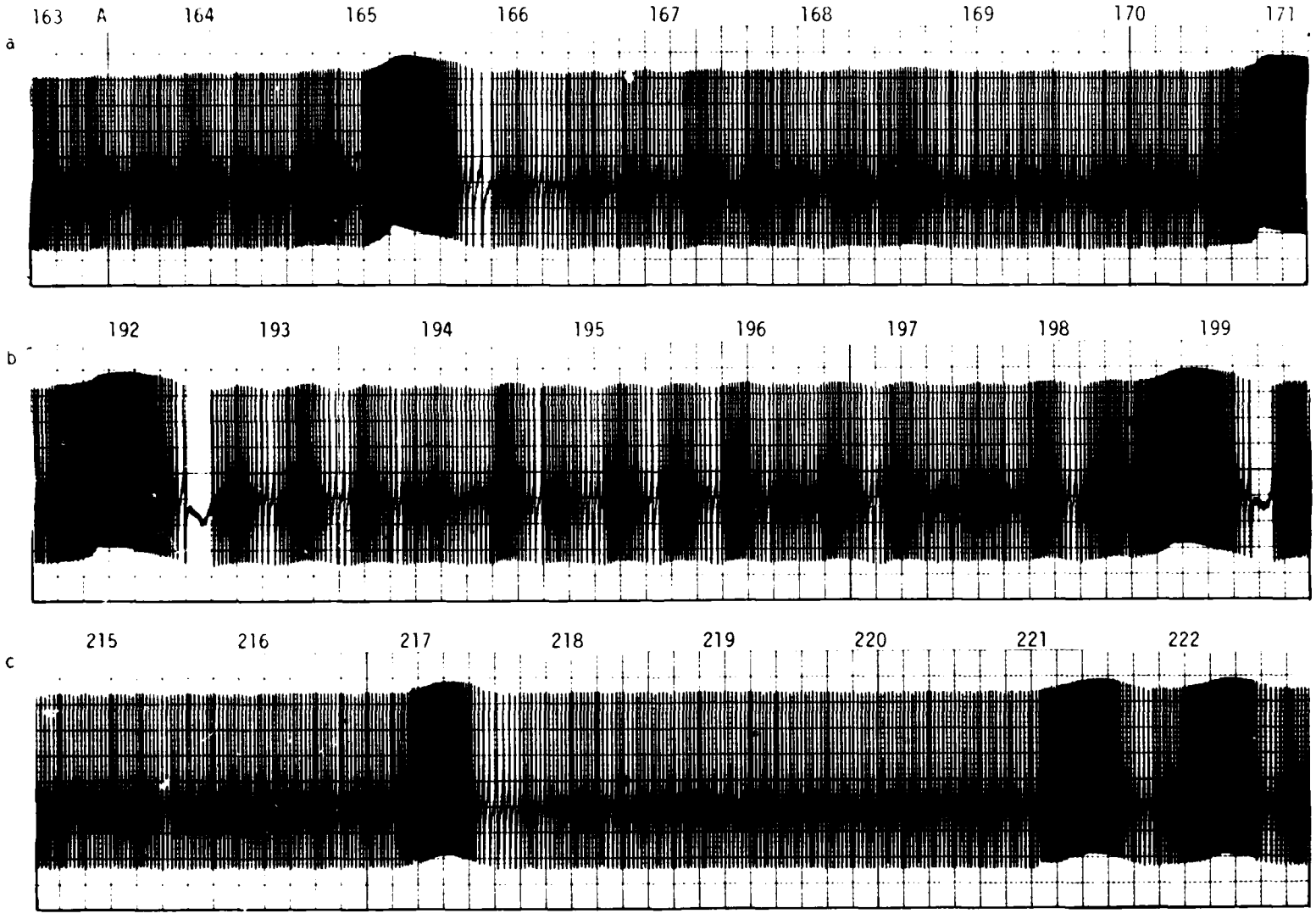


Figure C-3a-c. Post-exposure period of cell studied 18 June 1980.

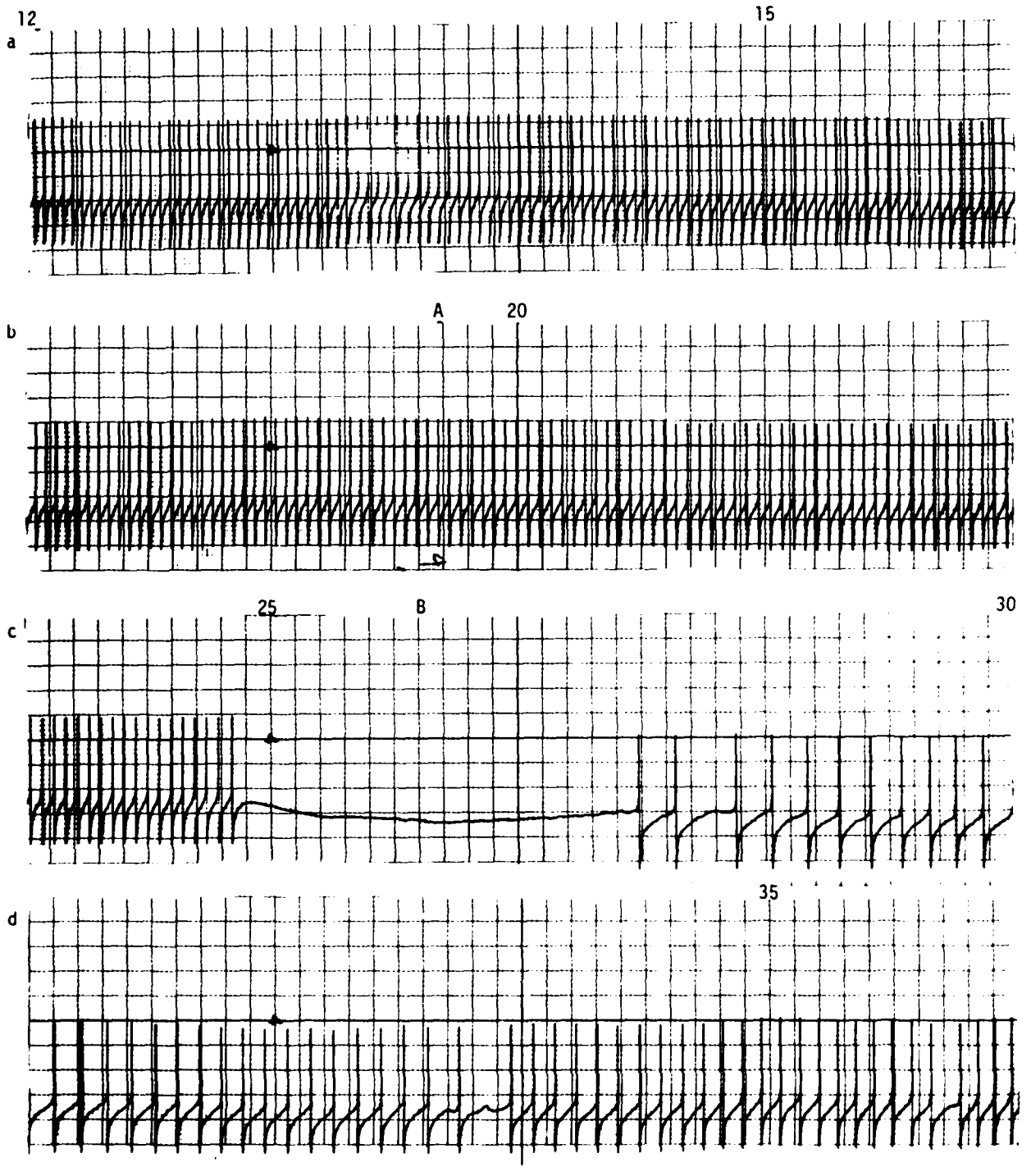


Figure C–4a-d. Baseline, first exposure, and first post-exposure periods of cell studied 7 May 1980.

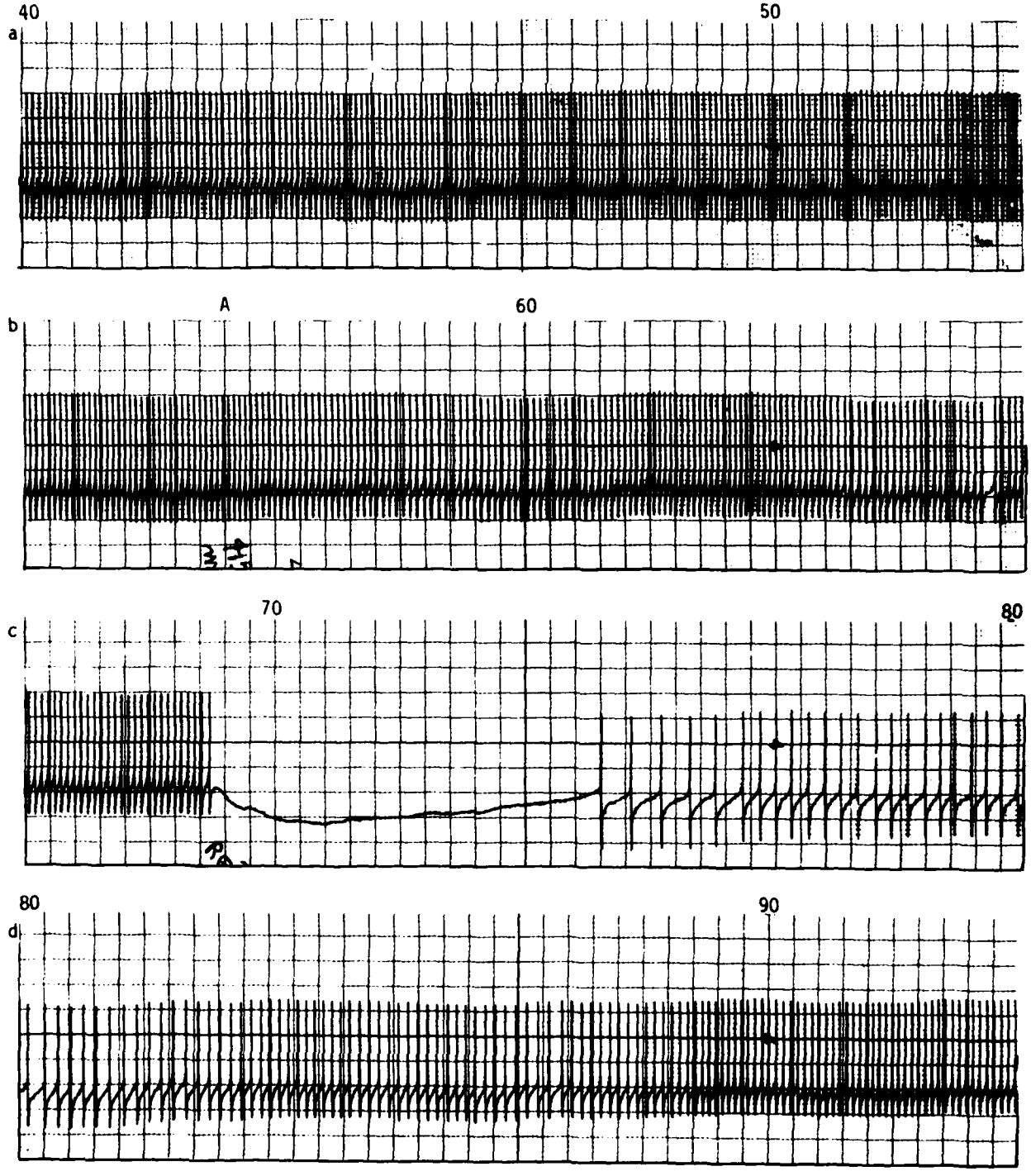


Figure C-5a-d. First post, second exposure, and second post-exposure periods of cell studied 7 May 1980.

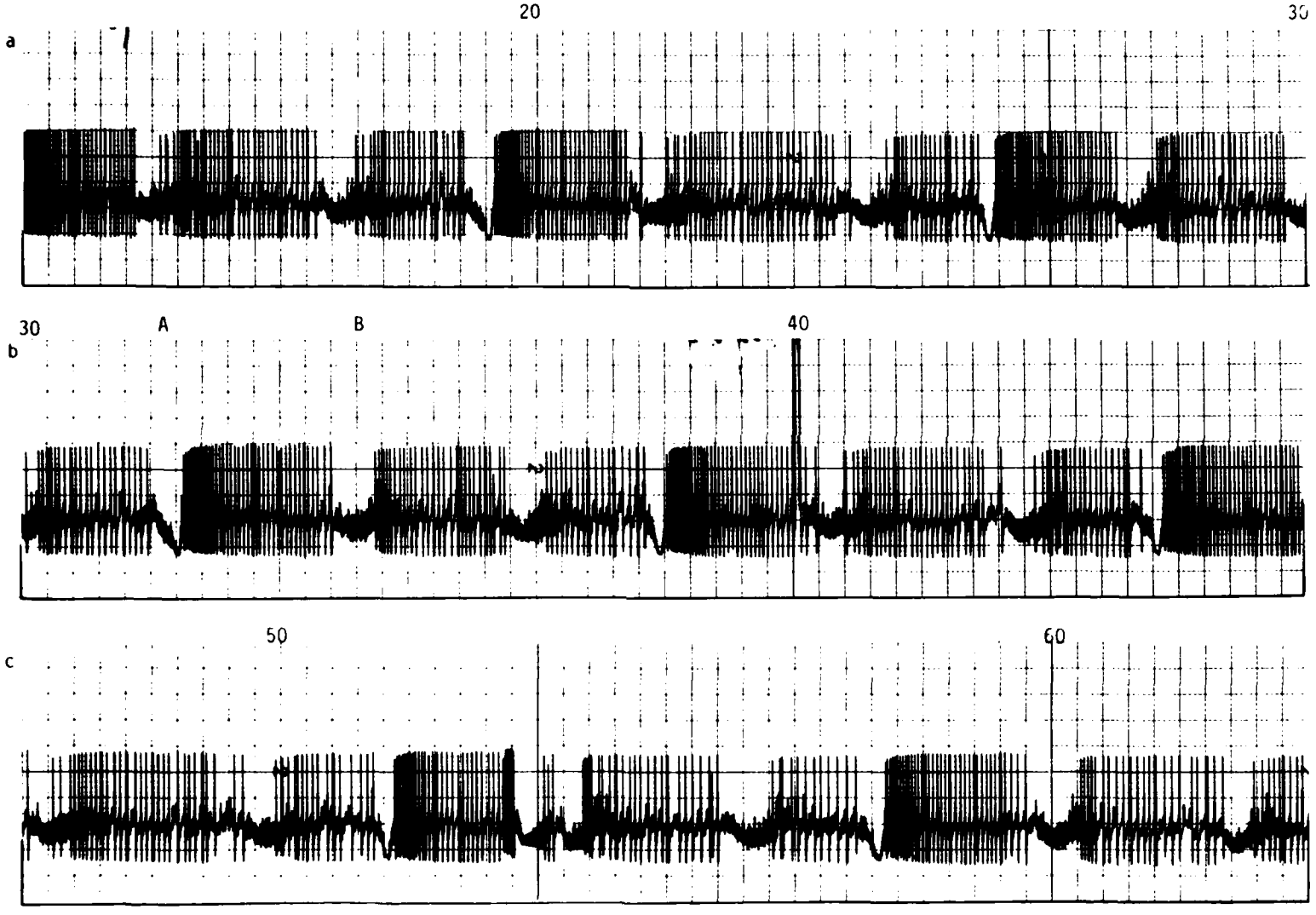


Figure C-6a-c. Baseline period of cell studied 17 July 1980.

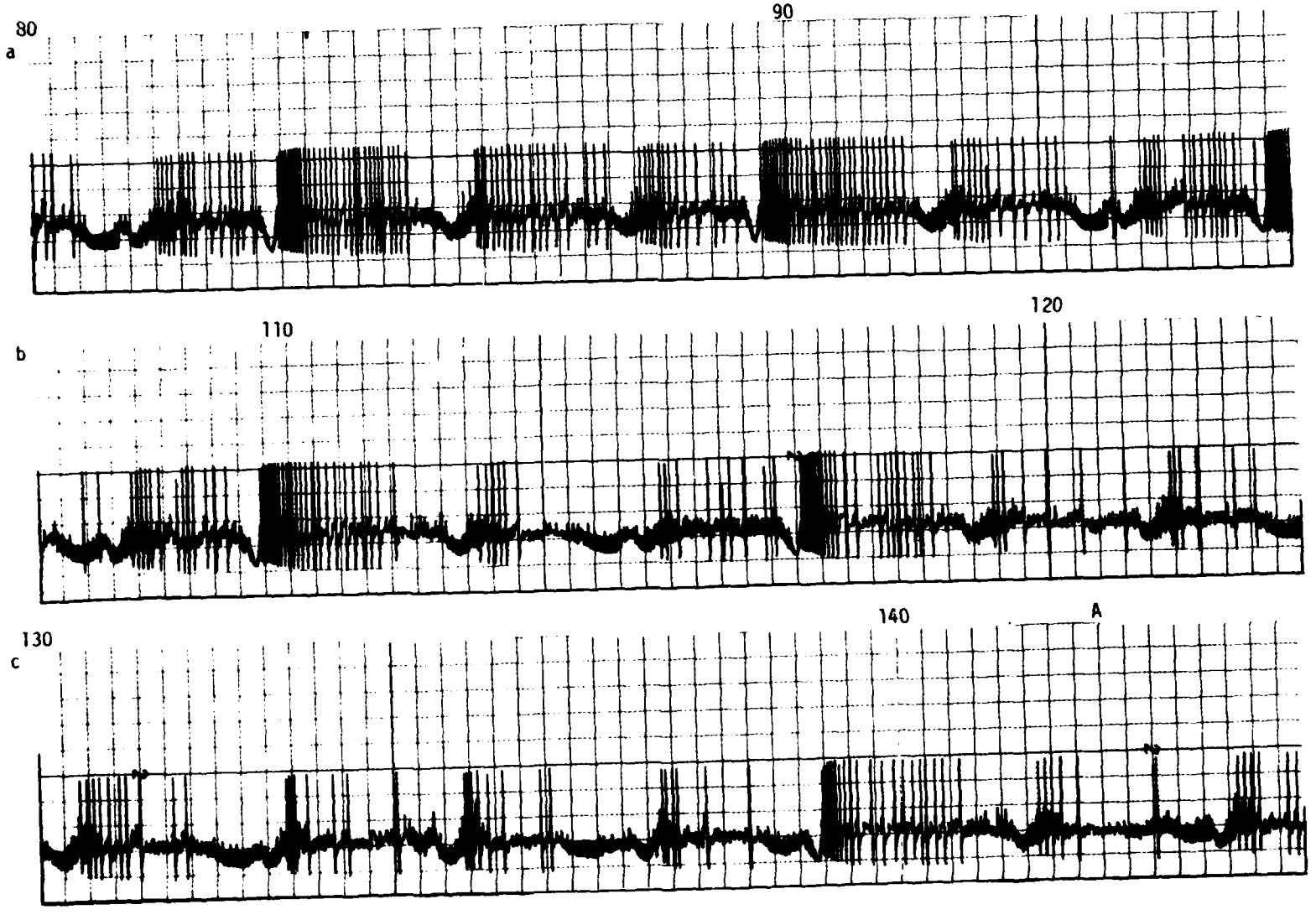


Figure C–7a-c. First exposure period of cell studied 17 July 1980.

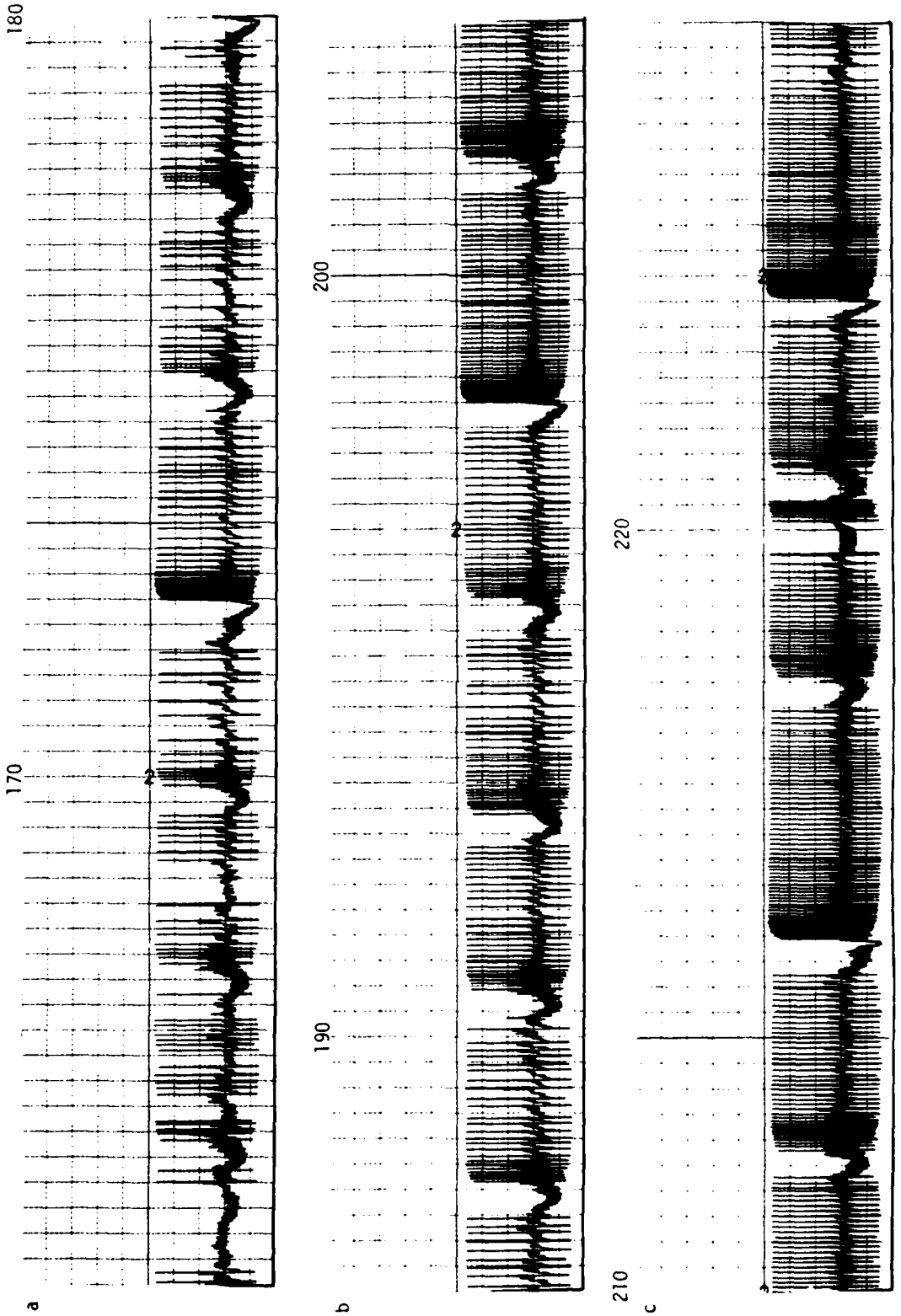


Figure C–8a-c. First post-exposure period of cell studied 17 July 1980.

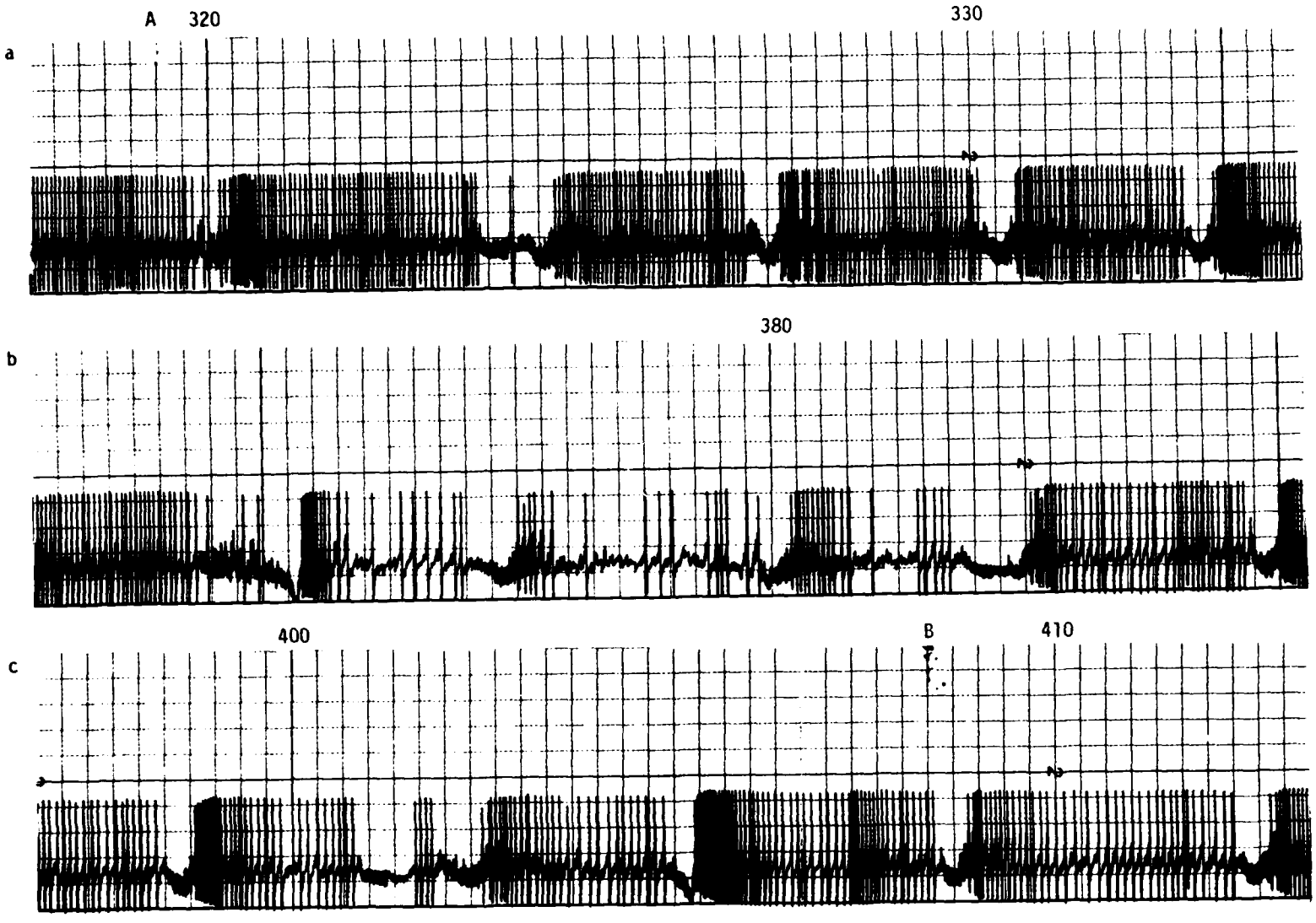


Figure C-9a-c. Temperature response of cell studied 17 July 1980.

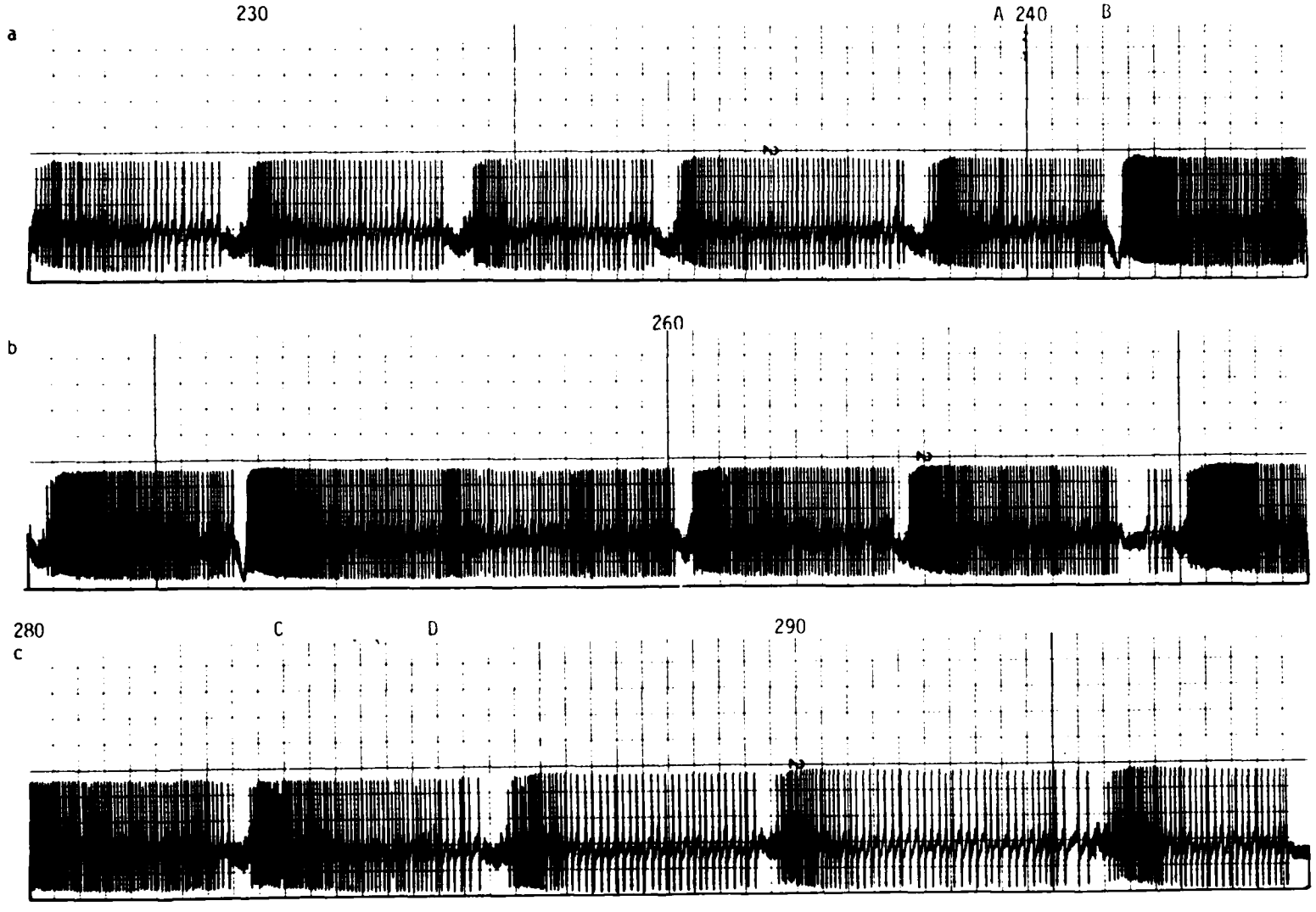


Figure C-10a-c. Second exposure period of cell studied 17 July 1980.

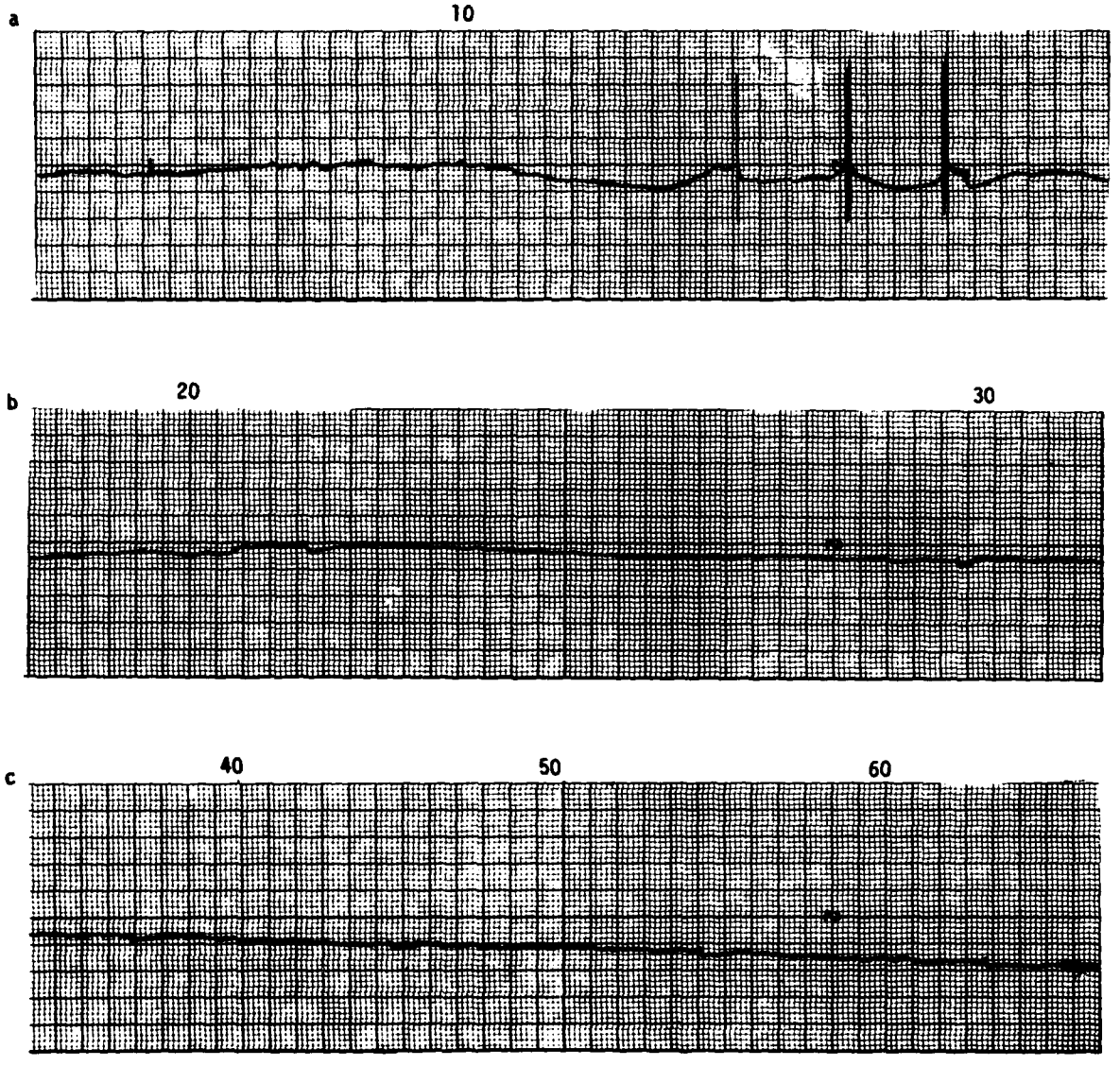


Figure C-11a-c. Baseline period of cell studied 23 September 1980.

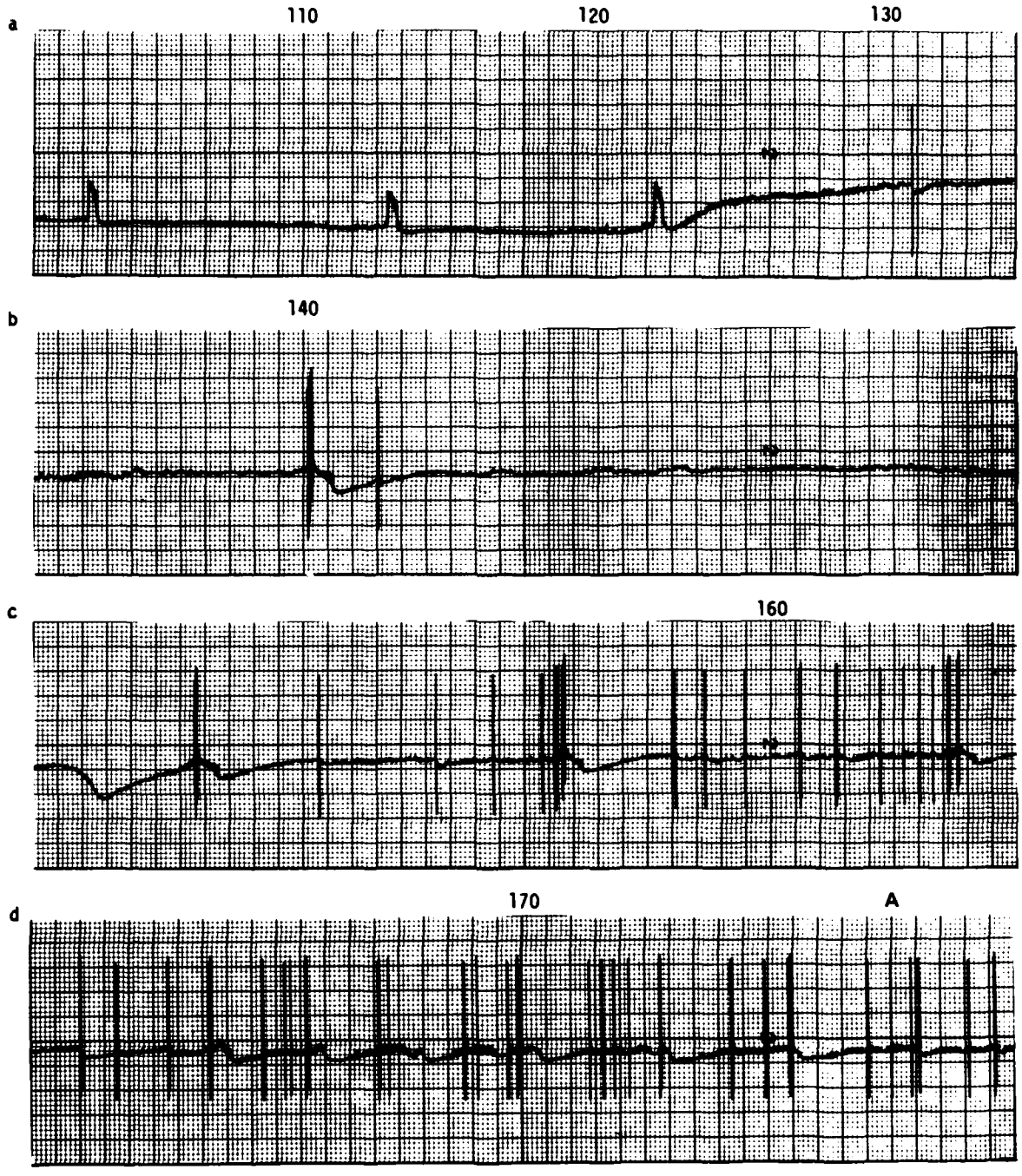


Figure C-12a-d. Exposure period of cell studied 23 September 1980.

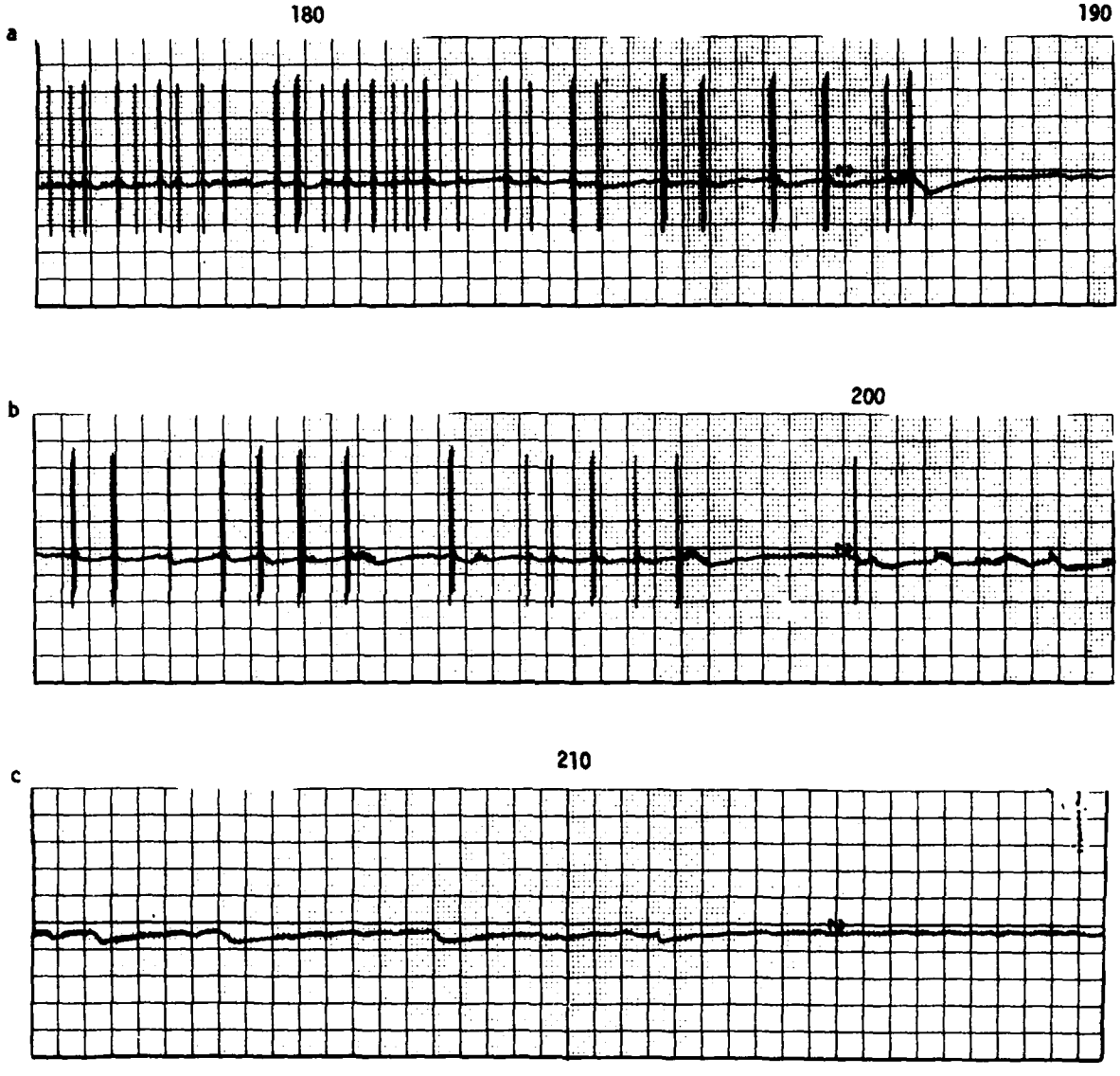


Figure C–13a-c. Post-exposure period of cell studied 23 September 1980.

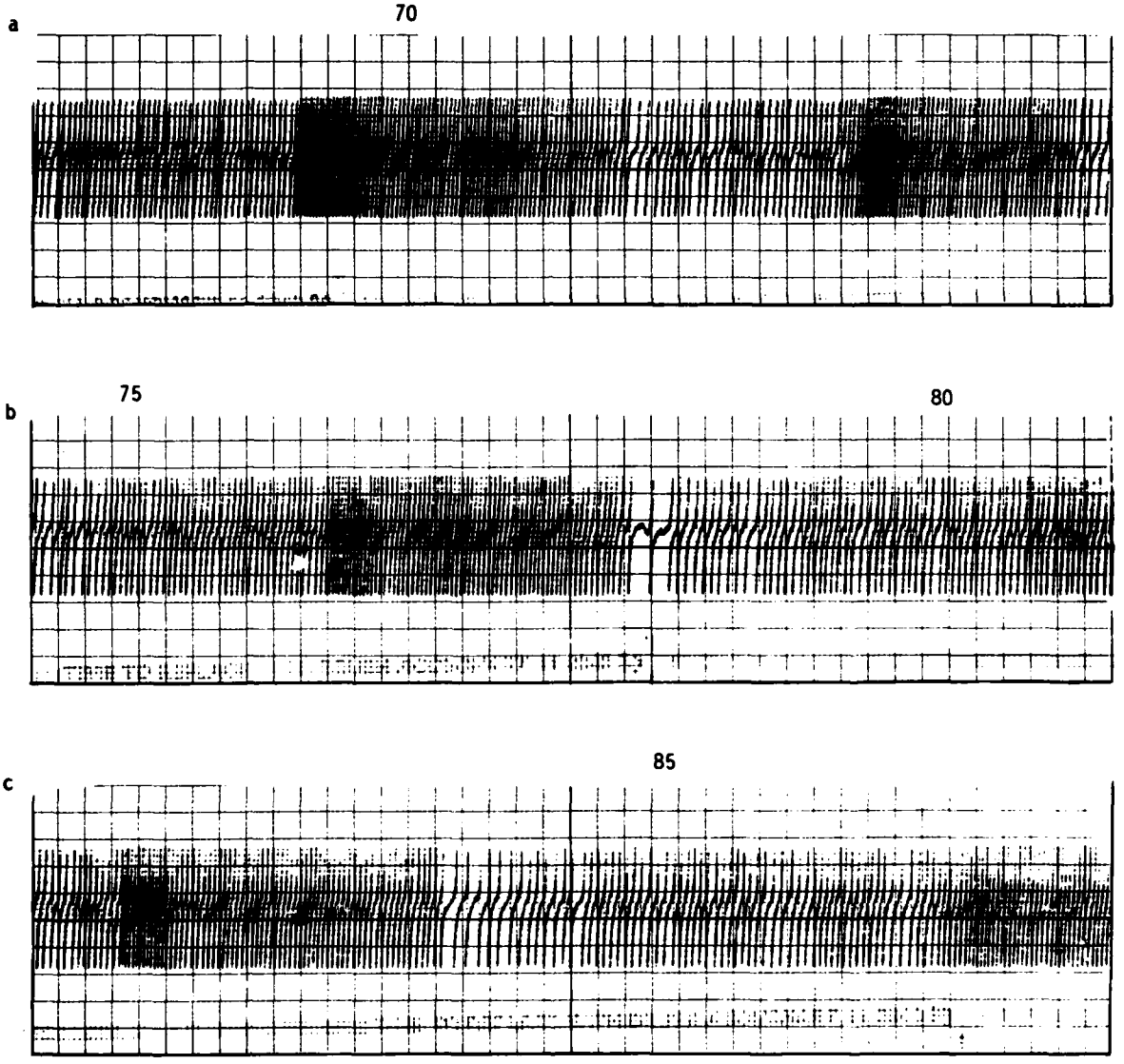


Figure C-14a-c. Baseline period of cell studied 27 January 1981.

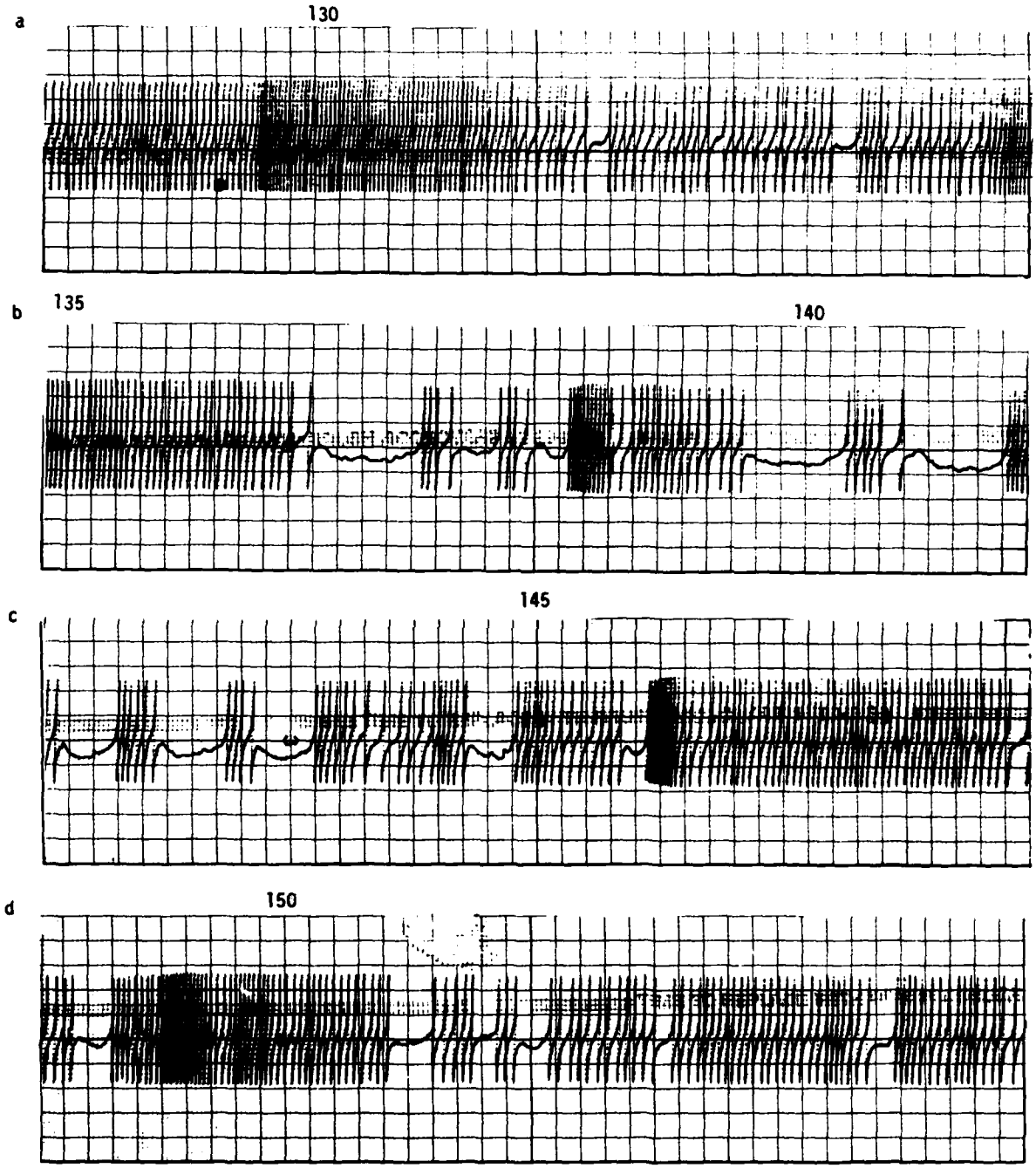


Figure C-15a-d. Exposure period of cell studied 27 January 1981.

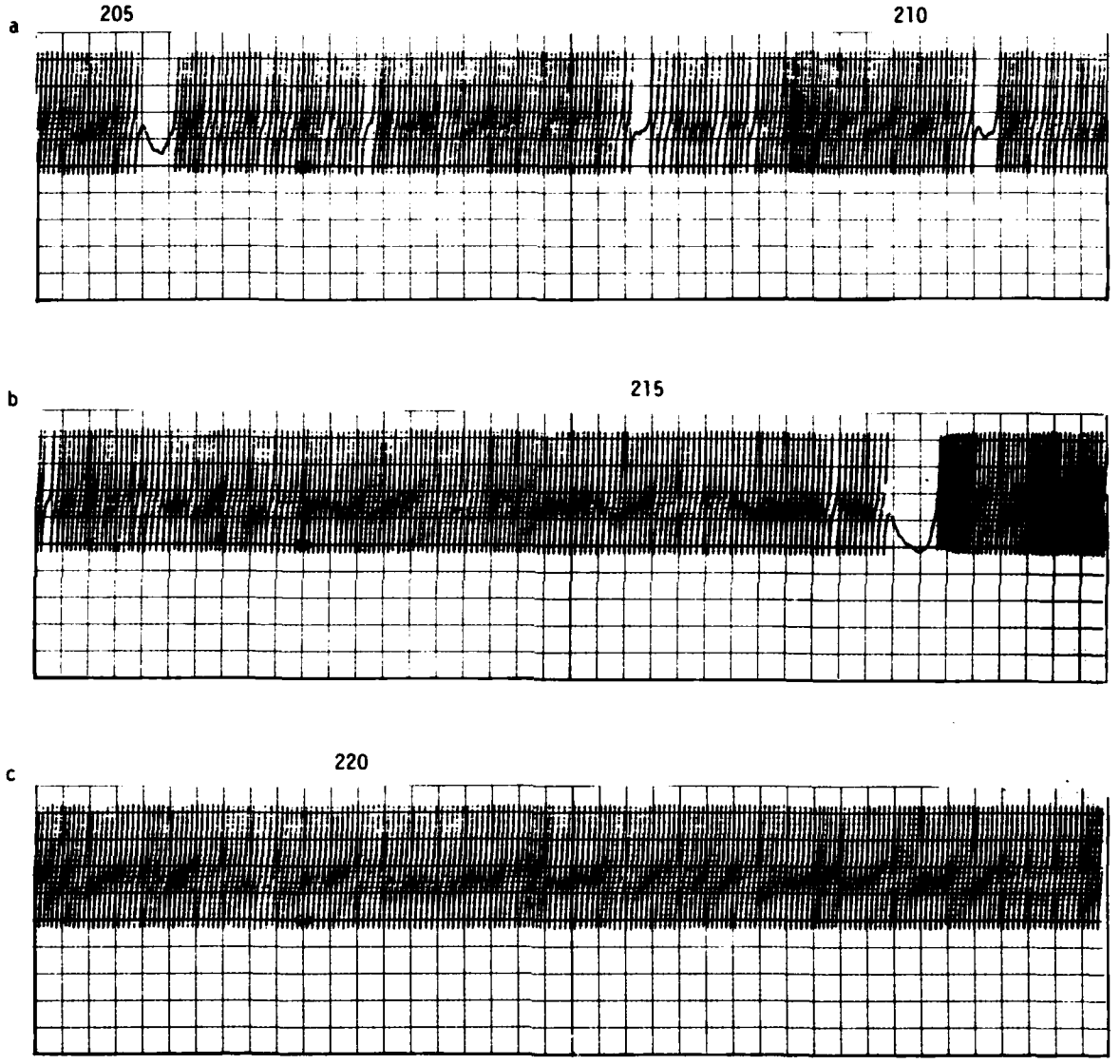


Figure C–16a-c. Post-exposure period of cell studied 27 January 1981.

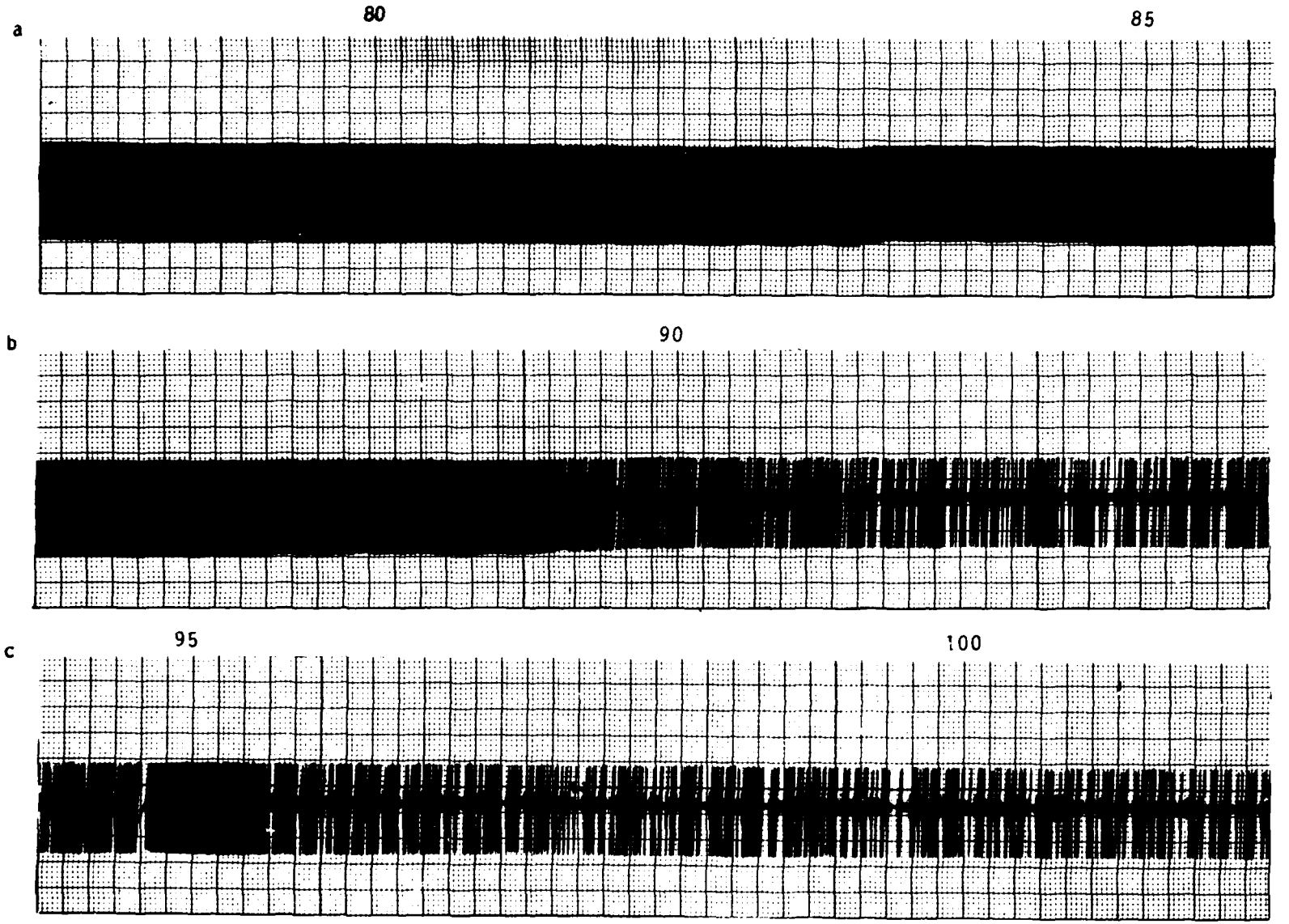


Figure C-17a-c. Exposure period of cell studied 29 January 1981.

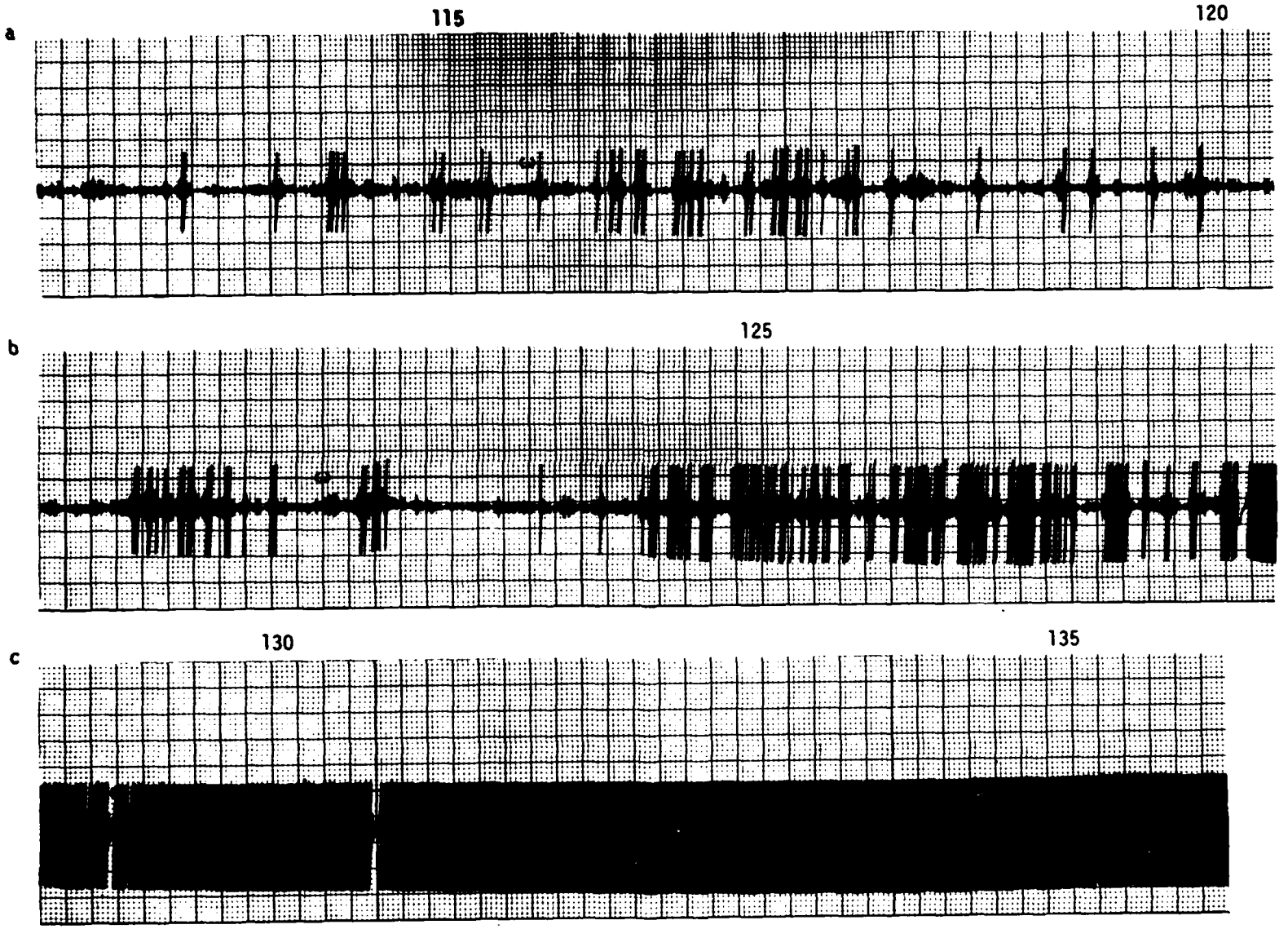
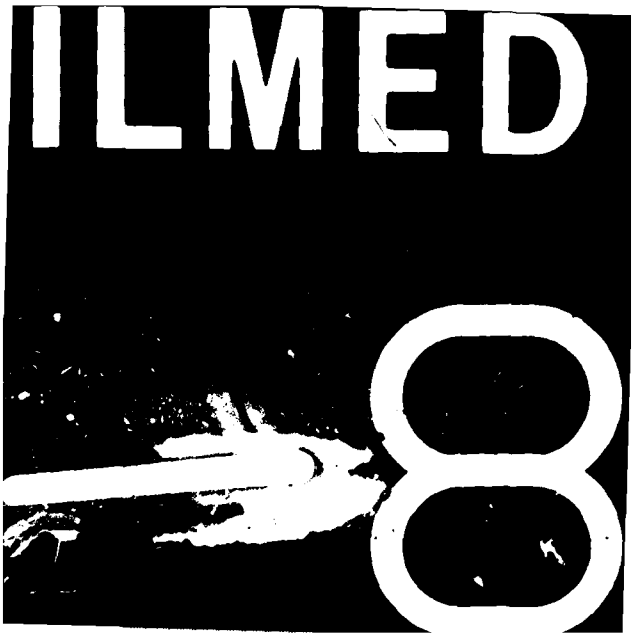


Figure C-18a-c. Post-exposure period of cell studied 29 January 1981.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